

ACTA ENDOCRINOLOGICA

SUPPLEMENTUM 6 (VI)

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STUDIES ON
PARATHYROIDAL FUNCTION IN RELATION
TO HORMONAL INFLUENCES AND
DIETETIC CONDITIONS

BY

BENGT ENGFELDT

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EINAR MUNKSGAARD
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BENGT ENGFELDT

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IN RELATION TO HORMONAL INFLUENCES
AND DIETETIC CONDITIONS

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SUPPLEMENTUM 4 (III).

FROM THE PATHO-ANATOMICAL DEPARTMENT,
KAROLINSKA INSTITUTET, STOCKHOLM

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TO THE MEMORY OF MY FATHER

P R E F A C E

The present investigation has been carried out in the Patho-Anatomical Institution of Karolinska Institutet during the years 1947—1950.

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Stockholm in November 1950.

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INTRODUCTION

The present work, based on experiments on animals, deals with the regulation of the parathyroidal function. It comprises morphological and cytological investigations and parallel with them chemical studies of blood and urine. The author's interest has been directed especially upon the problem of finding an accurate method for the morphological registration of the changes in the functional state of the parathyroids. Primarily the investigation was directed on the dependence of the parathyroidal function on other endocrinous glands, and especially on the anterior lobe of the pituitary. A number of problems concerning the importance of diet for the parathyroidal function have also been investigated.

Chapter 1.

SURVEY OF THE LITERATURE.

I. *The development of the theory of the parathyroidal function.*

The parathyroids were described for the first time in 1880 by Ivar Sandström of Uppsala. These glands had been observed earlier, however, as Sandström himself stated, but they had then been regarded as accessory thyroid glands. Sandström studied and described the parathyroids both in man and in a number of animals. He proposed the designation parathyroids for this organ, and this name has subsequently been generally accepted. His investigations were only published in Swedish, however, and therefore failed to arouse general attention.

The importance for the system of the parathyroids was suggested for the first time by Gley, who rediscovered the parathyroids in 1891 and showed experimentally that they were of decisive significance for the death-rate following thyroidectomy. Gley and, *inter alios*, Vassale and Generali (1900) connected the parathyroids with the tetany syndrome. In investigations in 1898 A. ver Eecke showed that the excretion of phosphate in the urine decreased after thyroidectomy, and this author believed that this was due to a reduced thyroidal function.

Erdheim (1906) observed changes in the teeth of rats after removal of the parathyroids. He showed that calcium was not deposited to a normal extent in the growing tooth in parathyroidectomized rats, and owing to this observation

attention was directed to the connection between the metabolism of calcium and the parathyroids. Mac Callum and Voegtlin (1906) observed that calcium might check the symptoms of parathyroprival tetany. In 1909 these workers demonstrated a reduced blood calcium rate after parathyroidectomy. They also showed that extract of parathyroids alleviated symptoms of tetany. Thus the connection between the parathyroids and the calcium metabolism was established. In a number of works, published in 1911 and later, Greenwald showed that the excretion of phosphorus dropped after parathyroidectomy and further found an increase in the blood phosphorus.

At the same time as the hypoparathyroidism syndrome was being studied, facts had emerged indicating that an enlargement of the parathyroids might be connected with certain bone diseases. Thus Askanazy (1904) described a case with bone changes and at the same time a tumour close to the thyroid gland. Subsequently the author pointed out that this tumour might have proceeded from the parathyroids, and he also suggested that the tumour might have had some connection with the bone disease.

Erdheim (1907) carefully investigated the parathyroids in a number of cases of osteomalacia and in some of them found an enlargement of the organ and in the other cases histological signs of hyperplasia. He assumed that in these cases the hyperplasia was a secondary phenomenon. Enlargement of the parathyroids has subsequently been demonstrated in a number of conditions which are directly or indirectly connected with the skeleton. Thus, in a comprehensive study on rachitis based on experiments, Erdheim (1914) reported enlargement of the parathyroids in rachitic animals. The same change was noted in Recklinghausen's disease, multiple myeloma, and skeletal carcinosis. At first it was generally considered, in agreement with Erdheim's conception concerning osteomalacia and rachitis, that the enlargement of the parathyroids was secondary to the bone changes, but gradually a doubt emerged as to whether this was always the case.

In 1915 Schlagenhauer suggested surgery in cases where only one gland was hypertrophic. This author was not disposed to explain such changes as secondary. In 1925 Felix Mandl, of Vienna, published the first case of osteitis fibrosa generalisata in which a parathyroidal tumour was extirpated. This procedure resulted in an immediate improvement. With this a very important advance had been made, and the understanding of the function of the parathyroids was considerably widened. In view of the striking improvement which set in after the removal of a parathyroidal tumour in patients with osteitis fibrosa generalisata, the conception that the parathyroidal changes were of a compensatory nature could no longer be maintained; on the contrary, it was obvious that the bone changes were secondary to a disturbance of the parathyroidal function.

At about the same time American workers had arrived at the same result. They had attacked the problem from the experimental physiological angle. Mac Callum and Voegtlin have been mentioned earlier. These authors showed that dogs with hypoparathyroidism had low serum calcium rates, and that tetany symptoms could be checked with injections of calcium (1908, 1909). Independently of each other, Hanson (1924) and Collip (1925) extracted the active substance from parathyroids. In 1925 Collip succeeded in demonstrating that dogs which had been given large doses of parathyroid extract presented a markedly raised serum calcium level. In the same year Greenwald and Gross showed that parathyroid extract gives rise to an increased excretion of calcium and phosphorus, which results in a negative calcium balance. Proceeding from these findings, the American physicians Hannon, Shorr, McClellan and Du Bois (1930) demonstrated an over-production of parathyroidal hormone in a patient with osteitis fibrosa generalisata, and after repeated operations a parathyroidal tumour was found in the mediastinum. For this clinical picture Barr, Bulger and Dixon (1929) proposed the name »hyperparathyroidism«.

II. *The systemic action of the parathyroid hormone.*

As has been indicated above, owing to the work of Hanson and Collip an active extract of parathyroids became available, and this provided a fair opportunity of investigating more in detail the importance of the parathyroids and the mechanism of the hormonal action. These problems have been studied by a number of single workers and teams (*inter alios*, Collip and his associates, Greenwald *et al.*, Albright *et al.*, Tweedy *et al.*, Salvesen, Shelling), and as a result of their observations we now have a fairly clear picture of how the metabolism of calcium and phosphorus is influenced by various functional conditions of the parathyroids.

The blood calcium is present practically exclusively in plasma. One portion is diffusible, the other portion is bound to protein and is non-diffusible. The diffusible portion is entirely or practically entirely ionized (*inter alios*, Thomson and Collip, 1932). The amount of ionized calcium is dependent in the first place on the relation between the concentrations of calcium and plasma protein (Mc Lean and Hastings, 1935).

The relation between certain ions in the blood and tissues, *inter alia* calcium, has proved to be decisive for the irritability of the musculature (Loeb, 1900, and others).

Phosphorus occurs in the system especially in the form of orthophosphoric acid and its derivatives. Orthophosphoric acid is found free or bound to various organic compounds (*inter alios*, King, 1946). The free phosphate, which is also termed inorganic phosphate, can be supplied to the blood by means of the increased splitting of organic phosphorus compounds or by decreased esterification of phosphate. A rise in blood phosphorus may also be due to calcium phosphorus compounds being released from the skeleton or by ingestion of phosphorus.

If the parathyroids are removed from a mammal, renal elimination of phosphate declines rapidly, and the blood phosphorus level rises. At the same time the blood calcium drops; and the excretion of calcium decreases. After para-

On the injection of large and repeated doses of parathyroid hormone the animals develop gastro-enteric symptoms, thromboses, anuria, coma, and soon die (Heuper, 1927). Similar pictures have been described in cases of acute hyperparathyroidism in man (Mellgren, 1943). On autopsy lime deposits are often found in various organs, especially in the liver and kidneys.

In addition to the above-described changes in the chemistry of the body fluids after treatment with parathyroid hormone, there are also bone changes. It has been established that the skeletal changes associated with osteitis fibrosa generalisata may be evoked by means of experimental hyperparathyroidism in suitable animals. (*Inter alios*, Jaffe, Bodansky, Blair, 1930—1932).

III. *The mechanism of parathyroid hormone action.*

The changes in the calcium phosphorus metabolism associated with different functional conditions of the parathyroids are now well known and no longer controversial. On the other hand, different opinions have been advanced in respect of how the hormone evokes these changes. On the whole there are two separate views on this question. According to one, the hormone affects the primary calcium metabolism by acting on osseous tissue, and the changes in the blood chemistry are secondary (Selye, 1932, 1942, Collip and Thomson, 1932, McLean and Bloom, 1937, Jaffe, 1933, Wilton, 1934, 1946). Thus, according to this theory, the parathyroid hormone rules the rate and direction of the mineral exchange between bone and body fluids by means of changes in the metabolism of the bone cells (McLean and Bloom, 1937). Wilton (1934, 1946) has studied this question exhaustively, both in cases of experimental hyperparathyroidism in guinea-pigs and in cases of osteitis fibrosa generalisata in man, and on the basis of his investigation he has arrived at the opinion that, by direct or indirect action on the bone cells, the excess hormone prevents the latter from reaching full maturity,

whereby their capacity to produce the enzyme which precipitates bone salt declines. If the excess hormone persists for any considerable period, the bone concerned must be replaced with lower differentiated bone tissue, and the bone becomes deficient in calcium salts. In his experimental investigations Wilton also observed that, in the case of an excess of parathyroid hormone, apart from a decrease in the calcium salts in the bone, the collagenous substance also disappears.

The second view concerning the action mechanism is, that the primary factor is the effect of the hormone on the electrolytic balance of the body fluids, and the bone changes are considered to be secondary to the changes in the blood chemistry (*inter alios*, Albright and his associates).

According to this theory, the phosphate in the body fluids is affected, in the first place, in such a way that it is more readily eliminated by the kidneys, and as a result the blood phosphorus decreases, and the body fluids become less saturated in respect of the factors which form part of the equilibrium constant that determines the calcium-phosphorus values. On this there follows an increased absorption of calcium-phosphorus salts from the bone absorbing surface, and the blood calcium increases. When equilibrium is again reached, there would not be any more changes if it were not that a higher calcium content leads to an increased renal excretion of calcium. The loss of calcium thus arising is a factor which tends to lead to under-saturation in the body fluids, so that unless calcium is supplied with the food the mineral reserve in the skeleton must make good the deficit (Albright, 1948).

During a long course of years it has been discussed whether the parathyroid hormone acts primarily on the bone tissue or on the metabolism of phosphorus, but it has been difficult to arrive at any definite conclusions, viz. owing to the close connection between the metabolism of calcium and that of phosphorus. Later investigations of the systemic action of parathyroid hormone, especially the conditions in the absence of functioning kidneys, have contributed somewhat towards solving the question. (*Inter alios*, Ellsworth and Futcher,

1935, Neufeld and Collip, 1942, Ingalls, Donaldsson, Albright, 1943). Proceeding from the observations made in more recent years, it now seems possible to combine the two views on the mechanism of the parathyroid hormone action. This seems to have a primary effect on the phosphorus metabolism in such a way that the renal excretion of phosphate increases, but the hormone also appears to have a direct effect on bone tissue, leading to disturbances in the metabolism of the bone cells and subsequent decalcification. It seems, however, as if the capacity of the parathyroid hormone to mobilize calcium is greatly reduced in the absence of kidneys. In appraising this phenomenon it must be borne in mind that, if the kidneys are put out of function, this may give rise to extensive changes in the chemistry of the body fluids.

IV. *The regulation of the parathyroidal function.*

In the regulation of the parathyroidal function, generally speaking three different components may be conceived as being of significance. Thus the function may be expected to be affected hormonally (A); it may also possibly be affected by changes in the metabolism of minerals (B); and, finally, nervous stimulations (C) may be of significance for the function.

A. *Hormonal regulation.*

1. The connection between the pituitary gland and the parathyroids.

The question whether the parathyroidal function is affected by hormonal factors has long been discussed, and special experimental studies have been made in respect of a possible connection between the pituitary gland and the parathyroids. Attempts have been made in various ways to prove that the anterior lobe of the pituitary produces a parathyrotropic hormone, and the problem has been attacked in the first place morphologically, i. e. by trying to produce atrophy or hypertrophy respectively by hypophysectomy or treatment with extract of pituitary gland. Further, attempts have also been

made to demonstrate such changes in the chemistry of the blood as would indicate either a reduced or an increased parathyroidal function.

With regard to the morphological effect of hypophysectomy on the parathyroids, Ascher pointed out as early as in 1912 that no material changes in the parathyroids could be observed in hypophysectomized dogs. Nor did Collip (1934), who used rats in his experiments, consider that there was any definite atrophy. However, a number of workers state that they have observed signs of atrophy of the parathyroids after hypophysectomy (Smith, 1927, Koster 1930, White, 1933, Desclin, 1943). Houssay and Sammartino (1933) noted changes in the parathyroids in hypophysectomized dogs in about $\frac{2}{3}$ of the instances. They reported atrophy of the cells of the parathyroids, with reduced amounts of cytoplasm and increased connective tissue. The changes were often local and separated by areas normal in appearance. These authors considered that the changes might be due to the loss of a parathyrotropic factor, but that they might also be the result of a change in nutritional conditions. Baker (1942), who worked with monkeys, came to a similar conclusion. Volume determinations of the cells and nuclei indicated a slight atrophy after hypophysectomy. However, the appearance and distribution of the cells were normal, and therefore the author was of the opinion that if there was a parathyrotropic factor, it was not of the same essential significance for the function as the factors which stimulate the thyroid and adrenals.

The evidence as to the effect of preparations of the anterior lobe of the pituitary gland on the morphology of the parathyroids is also conflicting. In 1933 Anselmino, Herold and Hoffman stated to have produced an enlargement of the parathyroids in rats by means of injections of an extract from the anterior lobe of the pituitary gland of cattle. Doses of 30—50 mg. for 3—4 days led to an enlargement of the parathyroids by 100—300 % estimated from sections from the glands. These investigators also described morphological changes in the parathyroids. They stated that the light chief

cells predominate, that the oxyphilous cells disappear, and that the gland becomes hyperemic. Further, they observed an enlargement of the parathyroids in rabbits, but not in cats, dogs or guinea-pigs. These animals, however, did present the histological changes which were demonstrated in the parathyroids of the rats treated with pituitary gland. These workers interpreted their observations as indicating the occurrence of a parathyrotropic factor in the anterior lobe of the pituitary gland. Other authors have described similar changes in the parathyroids after treatment with extract of pituitary gland (Hertz and Kranes, 1934, Ham and Haist, 1939, Blumenthal and Loeb, 1942). Kemp and Marx (1937) observed changes in the parathyroids of dwarf mice which had been treated with a preparation of growth hormone. They considered that these changes might indicate a certain stimulating effect of the preparation on the parathyroids.

Evans, Simpson and Li (1948) produced gigantism in rats with pure growth hormone. In the animals treated the parathyroidal cells were possibly somewhat more abundant in cytoplasm than normally, and a more pronounced pseudo-alveolar structure was also observed.

However, several investigators have not been able to find any changes in the morphological aspect of the parathyroids in animals which had been given pituitary extract (Thomas and Cushing, 1939, Houssay *et al.*, 1933, Cattaneo, 1938). Campbell and Turner (1942) examined the weight of the parathyroids and the frequency of mitosis in rats treated with various amounts of pituitary extract up to a daily dose of 4,460 mg. They then saw the effect of all known pituitary hormones but did not note any definitely increased frequency of mitosis. From this these workers drew the conclusion that it is very improbable that a parathyrotropic hormone plays any part in the metabolism of calcium.

In morphological investigations of the pituitary gland of rats after parathyroidectomy a decreased bulk of the whole gland, including the anterior lobe, has been demonstrated in

female rats, while, on the other hand, male rats showed no change in the size of the pituitary gland (Brolin, 1948).

Experimental studies as to whether changes in the calcium and phosphorus contents of the blood are associated with different functional states of the pituitary gland, have also been carried out by a number of workers.

In the majority of papers dealing with the effect of hypophysectomy on the calcium content of the blood it is stated that no definitely established changes take place (Houssay and Mazzocco, 1922, Gerschman, 1931, Marenzi and Gerschman, 1934, Wallace, 1935). However, certain workers have found a reduced calcium content after hypophysectomy (Riddle and Dotti, 1936, Shapiro and Zwarenstein, 1933), while some state that there was an increase in the blood calcium (Kusunoki, 1927, Collip, 1934 /uncertain/). Some investigators have carried out their experiments with the animals on a diet deficient in calcium, without being able to establish any change in the blood calcium after hypophysectomy (Anderson and Oastler, 1938, Carnes, Oesbold and Stoerk, 1943, Törnblom, 1949). On the other hand, a rise in blood calcium has been demonstrated after removal of the pituitary, thyroid and parathyroids as compared with thyroid-parathyroidectomy alone in rabbits which had been given a diet containing ample calcium but little phosphorus (Törnblom, 1949).

Carnes and his associates (1943) have pointed out the possibility that the pituitary gland contains a factor which increases phosphorus, and that, by way of the latter, the pituitary gland effects a change in the phosphorus of the blood. These changes in the blood phosphorus level can then in their turn influence the parathyroidal function. On the basis of his comprehensive investigations Törnblom has advanced support for this opinion.

No change has been found either as regards the effect of hypophysectomy on the blood phosphorus by a number of authors (Mazzacco, 1927, Kusunoki, 1927, Gerschman, 1931), while others have observed a depressed phosphorus level

(Kobayashi, 1931, Ichijo, 1934, Cannavò and Beninato, 1935, Anderson and Oastler, 1938, Jones and Shinowara, 1942, Törnblom, 1949, Li *et al.*, 1949). Only occasionally a raised blood phosphorus level has been recorded after hypophysectomy (e. g. Vernetti, 1939).

Investigations have also been carried out in respect of the calcium and phosphorus contents of the blood after the action of preparations from the anterior lobe of the pituitary gland. According to some authors, the calcium content of the blood exhibits no change after the injection of anterior lobe extract (Cannavò, 1932, Dixon, 1933, Snyder and Tweedy, 1941). On the other hand, a number of authors consider that they have demonstrated a definite rise in the calcium level as a result of the influence of anterior lobe extract (Anselmino, Herold and Hoffman, 1933, Gerschman and Marenzi, 1935, Shapiro and Zwarenstein, 1933, Friedgood and Mc Lean, 1937). A depression of the calcium level has also been claimed, however, as the result of treatment with anterior lobe extract (Teel and Watkins, 1929, Hogben and Charles, 1932). In rabbits without pituitary, thyroid and parathyroids, and kept on a diet abundant in calcium and scanty in phosphorus, Törnblom (1949) observed a drop in the blood calcium after treatment with anterior lobe extract, ACTH, and adrenal cortex extract.

Changes in the phosphorus content of the blood after treatment with pituitary extract have been recorded by a number of investigators. Under the conditions mentioned in the preceding paragraph, Törnblom (1949) produced an increase in the blood phosphorus. On the basis of his investigations, he drew the conclusion that the phosphorus-increasing effect of the pituitary gland is brought about via the adrenals. Treatment of hypophysectomized rats with growth hormone from the anterior lobe leads to a rise in the low phosphorus values to normal values (Li *et al.*, 1949). An increase in the blood phosphorus has also been observed by Cannavò, 1942, Gerschman and Marenzi, 1935, while others state that they have noted a depressed phosphorus level (Ko-

bayashi, 1939, Ichijo, 1934). Absence of change, or uncertain changes, in the blood phosphorus after treatment with anterior lobe extract have also been reported (Teel and Watkins, 1929, Thompson and Cushing, 1934, Friedgood and Mc Lean, 1937).

Morphological changes of the anterior pituitary lobe in man have been observed in a number of cases of primary hyperparathyroidism (Mellgren, 1943, Wilton, 1946). There were non-granular cells with ample thin, pale cytoplasm and small compact nuclei. These cells were compared by Wilton to foam cells.

Summary: If attempts are made to collocate the above results of the experimental investigations dealing with the connection between the anterior lobe of the pituitary gland and the parathyroids, the picture which emerges is hardly uniform. Both the morphological investigations of the parathyroids after hypophysectomy and after treatment with various pituitary extracts from the anterior lobe, and the investigations of the changes in the blood chemistry under the same experimental conditions have yielded divergent results. One group of findings which, however, can be ignored is that dealing with the conditions in birds and lower animals, since in them the follicle hormone has an obvious effect on the calcium metabolism as opposed to the conditions obtaining in mammals. So much can be said, however, that, judging by the statements in the literature, there is no definite evidence of a parathyrotropic factor in the anterior lobe of the pituitary gland. Some of the observations discussed above argue rather against the existence of a parathyrotropic factor. Thus the majority of investigations show an increase in blood phosphorus after treatment with pituitary extract and a reduction in blood phosphorus after hypophysectomy. These findings are hardly compatible with the presence of a parathyrotropic factor in the anterior pituitary lobe, since a direct stimulation of the parathyroids by a possible parathyrotropic factor ought to lead to raised blood calcium and reduced phosphorus rates, and further, the absence of the parathyrotropic factor ought to depress the blood calcium and raise the blood phosphorus.

2. The connection between the parathyroids and other endocrinous-organs.

There are a number of experimental investigations of the adrenals which might indicate a possible functional connection between them and the parathyroids. Blumenfeld and Clausen (1940) recorded the volume of the parathyroids in rats after removal of the adrenals. They state that the volume of the parathyroids was then somewhat reduced, but the difference was not fully statistically valid. The blood calcium was somewhat lower than in the controls. After treatment with adrenal cortex hormone (Eschatin), the parathyroids presented an increase in volume, and the blood calcium rose to normal values. These authors interpret their results as showing that extirpation of the adrenals leads to a reduced parathyroidal function rather than otherwise. On the other hand, Rogoff and Stewart (1928) found that the blood calcium rates of adrenalectomized dogs showed a rising tendency, and that their parathyroids were often enlarged. Tobin (1939) observed that, when the suprarenal glands had been extirpated on the 17th day of pregnancy, the rat feti exhibited enlarged parathyroids at full term. A disturbance in the formation of dentin resembling the changes which develop after treatment with parathyroid hormone was noted in rats after extirpation of the adrenals (Schour, Rogoff, 1936). Rats whose adrenals have been extirpated exhibit more marked calcemia and calciuria than normaly after treatment with parathyroid hormone (Pugsley and Collip, 1936).

Investigations which bear directly on the metabolism of calcium and phosphorus in different functional states of the adrenals are naturally also of interest in this connection. Thus Törnblom (1949) observed that simultaneously hypophysectomized, thyroidectomized and parathyroidectomized rabbits on a diet rich in calcium and deficient in phosphorus showed an increase in blood phosphorus and a reduction in blood calcium in substitution experiments with corticotropic hormone and also after treatment with adrenal extract. No rises — or only uncertain ones — in the inorganic phosphate in the blood

after adrenalectomy were observed by some workers (Lucke and Heckmann, 1938, Magyary-Kossa, 1942), while others have found definitely raised blood phosphorus rates (Helve, 1940, Huber, 1950). Quite recent investigations on rats have shown that normal animals treated with ACTH show a depressed phosphorus level. The same is true of hypophysectomized animals which are given ACTH (Gemzell and Samuels, 1950).

Investigations into the relation of the parathyroids to the gonads have shown that, under certain conditions, the parathyroids are larger in female than in male rats, and that pregnancy, and especially repeated pregnancies, lead to a cumulative enlargement of the organ. Lactation is naturally also of importance (Bodansky *et al.*, 1941). No effect on the parathyroids following castration has been observed. Estrone evokes a well-marked calcemia in pigeons, but this is not due to the parathyroids. Bastenie and Zylberszae (1939) state that estrogen depresses the mitotic activity in the parathyroids of female rats. Nathanson and his colleagues (1940) found that testosterone propionate stimulated the mitotic activity in the parathyroids of immature female rats. Campbell and Turner (1942) did not note any change in the number of mitoses in the parathyroids of fowls or rats after treatment with estrone, nor could they stimulate cell division in the parathyroids with testosterone.

Various functional states of the thyroid have proved to influence the calcium metabolism. The excretion of calcium in cases of myxedema is considerably below normal, and hyperthyroidism entails an increased excretion of calcium in the urine; further, there is evidence of increased bone absorption after prolonged hyperthyroidism. No definite changes in the calcium and phosphorus contents of the serum are observed, however (Aub *et al.*, 1929). The injection of thyroxin into rats leads to an increased excretion of calcium and a negative calcium balance (Pugsley and Anderson, 1934). Logan and his associates (1942) showed that thyroid treatment of thyroidectomized and parathyroidectomized dogs did not increase the excretion of calcium, nor affect the serum calcium,

an observation which might possibly indicate that the action of the thyroid on the calcium metabolism is brought about via the parathyroids. By treatment of rats with thiouracil and similar preparations, Malcolm *et al.* (1949) produced osteitis fibrosa generalisata pictures in the skeleton and considerable hyperplasia of the parathyroids.

Houssay and his associates (1933) observed that in dogs whose pancreases have been extirpated, the parathyroids present regressive changes. These changes are more pronounced in pancreatectomized and hypophysectomized animals than in animals which are only hypophysectomized, the blood calcium in these animals dropping and the serum phosphorus increasing. In cases of untreated diabetes mellitus there is an increased amount of inorganic phosphate in the serum, and the phosphate decreases after treatment with insulin (*inter alios*, Levine *et al.*, 1942). Mentha (1941) found hyperplasia of the parathyroids and also skeletal changes resembling osteitis fibrosa generalisata in dogs with experimental diabetes.

Milcou and Pitis (1948) state that the pineal body has a stimulating effect on the parathyroids. In rats treated with pineal body extract they found morphological changes in the parathyroids, which they interpreted as signs of over-activity.

Clinical and patho-anatomical observations in cases of functional, endocrinous disturbances have in some cases suggested a connection between the parathyroids and other endocrinous organs. For example, in a number of acromegalia cases changes in the form of hyperplasia or adenoma formations were present in the parathyroids, and cases of osteitis fibrosa generalisata were also observed concurrent with acromegalia. In this connection it is of interest, *inter alia*, that Hurxthal (1948) demonstrated that patients with active acromegalia present a raised serum phosphorus level. Even in Cushing's syndrome changes in the parathyroids have been described in some cases, but these are unusual (Luft, 1944).

Summary: As will have been seen from the foregoing the question of the connection of the parathyroidal function with endocrinous organs other than the pituitary gland has been

very incompletely elucidated, and the information available hardly throws any clear light on this question. It appears possible, however, that there is some form of functional connection between the parathyroids and the adrenals, Langerhans' islands and the thyroid, but our knowledge of the mechanism of such a connection is practically nil.

B. Regulation through changes in the mineral metabolism.

From what has been said about the functional mechanism of the parathyroids there may be reason to expect that, in the regulation of the parathyroidal function, apart from the hormonal factors, changes in the calcium and phosphorus contents of the body fluids may be of significance. Either an increased blood phosphorus, or a reduced blood calcium level, or both together, might then be thought to stimulate the parathyroids to over-activity. On the other hand, a reduction of the blood phosphorus or a raised calcium level may lead to under-activity.

Bergstrand (1921), who, *inter alia*, investigated the size of the parathyroids in a large postmortem material, was able to show that the parathyroids were often markedly enlarged in cases of chronic renal insufficiency. As has subsequently been demonstrated, these patients have raised blood phosphorus and normal or low blood calcium rates (Albright, 1937).

A number of investigators have made experimental studies of how the calcium and phosphorus contents in the food effect the amounts of these substances in the blood, and also how the parathyroids behave under different dietetic conditions. It has been possible to show with rats that the variations in the calcium and phosphorus contents of the food are to a certain extent reflected in the blood (Ham *et al.*, 1940, Carnes *et al.*, 1942), so that, for example, an abundant supply of phosphorus in the food leads to an increase in the blood phosphorus. Careful studies have been carried out of what is to

be considered a normal content of calcium and phosphorus in the food, *inter alios*, by Cox and Imboden (1936) and Duff and Bodansky (1941), who employed the »reproductive success« in albino rats as a criterion of an appropriate calcium and phosphorus content. Cox and Imboden (1936) examined 145 female rats during ten successive pregnancies and found that both absolute quantities of calcium and phosphorus and their mutual relation in the food affected the results. A calcium-phosphorus ratio of about 1 and a calcium level of about 0.5 % in the food gave the best results and proved optimal in respect of pregnancy and lactation in albino rats.

Thus it has been shown that changes in the calcium-phosphorus content of the food result in changes in the contents of these substances in the body fluids. Whether this circumstance is of any significance for the parathyroidal function has also been the subject of investigations, some of the more important of which will be briefly surveyed. Baumann and Sprinson (1939) put rabbits on a diet containing a great deal of phosphorus and but little calcium, which led to enlarged parathyroids. These workers found that their animals had low calcium and phosphorus rates in the blood, but the values lay within normal limits. Ham and his associates (1940) put rats on a diet poor in phosphorus for three weeks, after which the calcium and phosphorus in the blood were estimated. These animals had low blood phosphorus rates and exhibited no hypertrophy of the parathyroids. Using a diet containing small amounts of calcium they produced low blood calcium rates and a normal blood phosphorus level, and in this series the parathyroids were hypertrophic. Campbell and Turner (1942) were able to show that rabbits on a diet poor in calcium developed enlarged parathyroids. They also investigated how rats behave on different contents of calcium in the food and with varying supplies of vitamin D, and demonstrated that, with insufficient supplies of calcium and enough vitamin D, the enlargement of the parathyroids was not so considerable but still definite. Stoerk and Carnes (1945) have shown that a diet poor in calcium leads to hypocalcemia

and enlargement of the parathyroids in rats, while a diet poor in phosphorus and abundant in calcium leads to a reduction of the volume of the parathyroids. These workers have also investigated the effect of different diets on the parathyroids and serum calcium. The calcium-phosphorus ratio varied in these experiments from 15.0 to 0.03. It emerged from the results that there was direct proportionality between the logarithm of the calcium to phosphorus ratio of the diet and the concentration of serum calcium in adult rats, and that the logarithm of the calcium to phosphorus ratio of the diet is inversely proportional to the volume of the parathyroids. The volume of the parathyroids and the concentration of blood calcium varied inversely proportional and almost linear with a blood calcium content varying from 7.3 to 11.9 mg. %. The serum phosphorus did not exhibit an equally regular relation to the size of the parathyroids in these experiments. Törnblom (1949) showed that, on a diet deficient in calcium and abundant in phosphorus, rabbits developed an enlargement of the parathyroids and increased blood phosphorus. If hypophysectomized rabbits were put on the same diet, there was no rise in blood phosphorus, nor did these animals present any enlargement of the parathyroids. In a morphological study of the parathyroids in rats with rachitis, partly as a result of insufficient supplies of calcium and partly following on insufficient supplies of phosphorus, De Robertis (1940) found pictures indicating over-activity of the parathyroids in both cases, but more pronounced with low calcium diets.

Thus it appears clear that the calcium and phosphorus contents of the diet may affect the parathyroidal function. It has also been discussed in that connection whether it is the change in the blood calcium or in the blood phosphorus, or both changes together, which are of decisive importance. Of the investigators quoted above Ham *et al.*, Campbell and Turner and Stoerk *et al.*, consider that the hypocalcemia is essential for the hyperplasia of the parathyroids. On the other hand, Törnblom's investigations point perhaps rather to the

increased blood phosphorus as the decisive factor. Another findings which may indicate that the blood phosphorus is of importance is the result of experiments with parenteral administration of phosphate buffer, but there is no evidence as to whether the hyperplasia is due directly to the hyperphosphatemia or whether it is brought about by a possibly accompanying hypocalcemia (*inter alios*, Drake and his associates, 1937).

Summary: As appears from the investigations quoted above, changes in the calcium and phosphorus contents of the diet affect the amounts of these substances in the body fluids, so that e. g. at least within certain limits, a decreased supply of calcium leads to a decreased calcium content in the body fluids. In their turn changes in the calcium and phosphorus contents of the blood affect the parathyroids. It has not been fully elucidated whether it is the change in the content of either of these substances, or of both together, that is of decisive importance.

C. *The nervous regulation mechanism.*

There is only scanty information with regard to the question of whether a nervous regulation mechanism is of any importance for the parathyroidal function. Actually, however, the parathyroids can be replaced by transplanted glands (*inter alios*, Houghton and his associates, 1939), or by tissue cultures, which appear to function satisfactorily. From this the conclusion may probably be drawn that a possible nervous regulation mechanism is hardly of material importance for the function.

Chapter 2.

THE OBJECT OF THE PRESENT INVESTIGATION

In experimental investigations of questions dealing with the function of the parathyroids the most important methods comprised on the one hand, a morphological examination of the parathyroids, and on the other, estimations of the calcium and phosphorus contents of the body fluids.

By earlier authors morphological appraisements of the functional conditions of the parathyroids have usually been effected by means of determining the weight or volume of the organ, but such data are often inadequate.

The size of the nucleus and the size of the cell are not mutually independent variables, but under normal conditions a given celltype actually shows a definite correlation between the nuclear mass and the cytoplasmic mass (Heidenhain, 1919). This nucleus — cytoplasm ratio, however, is not invariable under all conditions; for example it may be changed when for some reason the cells accommodate themselves to an increased or decreased function.

During recent years very important advances have been made in respect of our knowledge about the formation of protein in the cell, e. g. in growth and secretion. Thus, by means of cytochemical studies, Caspersson and his associates (1941) proved the importance of the nucleic acids in this connection. Cells in which large amounts of cytoplasmic proteins are being formed exhibit, firstly, a large nucleolus containing ample ribose nucleotides, and secondly, the cytoplasm

in such cells contains similar large amounts of ribose nucleotides, with, in certain cases, the greatest concentration adjacent to the nucleus. These findings have been interpreted as showing that the nucleus should be considered the centre of protein formation in the cell.

As is well known, the parathyroid hormone is a protein, and, proceeding from what has been said above, there is thus reason to expect evident cytological changes to accompany enhanced or reduced hormone formation.

The writer has considered that one of the main objects of the present study is to secure an accurate procedure of morphologically recording the separate functional states of the parathyroids, which not only comprises volume determinations but also cytological examinations.

Recent advances in the field of chemistry have also placed at our disposal methods which make it possible to estimate very small amounts of phosphorus (Norberg, 1942).

In the study of the functional states of the parathyroids, investigations of the content of phosphorus and calcium in the body fluids are of considerable interest. However, the metabolism of both phosphorus and calcium is affected by a number of different factors, apart from the parathyroids, and it is not possible to draw definite conclusion as to an increased or reduced parathyroidal function from the changes in the concentration of these substances in the body fluids.

Hardly any attempts have been made previously in experimental investigations on animals to combine the blood chemical findings with more exhaustive morphological studies of the parathyroids.

Therefore the purpose of the present study is to investigate the parathyroidal function by means of a combination of morphological data, comprising both volume determination and cytological investigation, with blood analyses in respect of phosphorus and calcium by a method which, at least as regards the former, permits of reliable determinations with small amounts of blood — thus feasible in serial experiments during the course of the investigation.

The questions to be dealt with are the following:

1. Is there a parathyrotropic factor in the anterior lobe of the pituitary gland?
2. If this should not be the case, is there nevertheless any form of functional connection between the pituitary gland and the parathyroids?
3. How could such a connection conceivably be brought about?
4. Which factor or factors in the pituitary gland produce this connection?
5. Is there any functional connection between the parathyroids and the adrenals?
6. Is there any functional connection between the parathyroids and the pancreas?
7. Are the parathyroids functionally insufficient in hypophysectomized animals?

Chapter 3.

MATERIALS AND METHODS

Selection of experimental animals.

When selecting experimental animals, consideration must be paid, firstly, to anatomical, and secondly, to blood chemical conditions. The common laboratory animals in this country from which the choice has to be made, are rats, guinea-pigs and rabbits. As the investigation also involves hypophysectomies, and guinea-pigs are not especially suitable for that operation, the choice lay between rabbits and rats.

In respect of anatomical conditions, the widely varying topography of the parathyroids in rabbits had to be taken into account. This animal usually has, on each side of the throat, firstly, a parathyroid embedded in the thyroid, and secondly, another one outside the thyroid somewhere on the neck or in the mediastinum. White rats, however, have a certain advantage over rabbits in that for the most part they only have one parathyroid on each side, and this is situated close to or in superficial layers of the thyroid and usually is visible there to the naked eye. Apart from that, the serial sections of the throat organs necessary for volume determinations of the parathyroids are considerably more difficult to prepare in rabbits than in rats. For blood analyses, of course, fairly large amounts of blood can easily be collected from rabbits, and blood specimens can be taken continuously. On the other hand, there are difficulties in securing large quantities of blood from rats, at least if the animals are to survive. Estim-

ations of phosphorus, calcium and total protein form an essential part of the present study. The blood phosphorus is easily estimated, even in small specimens, and thus raises no great obstacle even when using rats. With the usual methods, however, blood calcium determinations require larger amounts of blood than can reasonably be taken from rats, if they are to survive, and therefore estimation of the blood calcium involves the killing of the animal. Thus in this respect rabbits would be more suitable as experimental animals. However, as regards the blood calcium, rabbits exhibit very great individual variations. Owing to this fact, Collip (1932) points out, rabbits are not suitable as experimental animals when it is a matter of calcium analyses. A secondary question which is of practical importance in an experimental study that may be expected to call for a large number of animals, is the possibility of breeding on a sufficiently large scale, and from that point of view naturally rats are to be preferred. I have myself been breeding albino rats since 1947, and all the morphological examinations have been carried out on rats of this breed. For some blood-chemical examinations rats were used which were supplied by the Gynaecological Department of the Karolinska sjukhuset, the reason for this being that those of my own breeding were not sufficient, as at times very many animals were needed. Only male rats were used in the experiments.

Dietary.

The animals were given a diet which was fully adequate in respect of vitamins and mineral, and they exhibited satisfactory growth and multiplication on this diet. For the standard diet, the following ingredients were used and were given to the animals in the form of a flour.

Maize flour	5 Kg.
Wheat germs	0.5 Kg.
Wheat gluten	2 Kg.

Casein sodium	1 Kg.
Evomin purum	1 Kg.
Sodium chloride	50 Gm.
Potassium chloride	50 Gm.
Magnesium sulphate	50 Gm.
Ammonium phosphate	115 Gm.
Ferric citrate	10 Gm.
Calcium carbonate	200 Gm.

Evomin purum is a vitamin preparation containing all the vitamins and growth factors found in yeast and also vitamin D. Each gram of the preparation contains, *inter alia*, at least 120 I. U. of B₁, 0.050 mg. of B₂ and 0.5 mg. of nicotinic acid and about 500 I. U. of D. This standard diet contains about equal parts of calcium and phosphorus, and the absolute calcium content is about 1 %. In the experiments where the animals were fed on diets abundant in calcium or in phosphorus, the amount of these elements in the diet was changed, while the other ingredients remained unchanged.

The animals were kept in steel-wire cages with sheet metal floors, which were covered with shavings. In order that specimens of urine might be obtained, the animals were put in cages with grating floors and with arrangements for collecting the urine and separating it from the excrements. The cages stood in a light, airy room at ordinary room temperature. For the animals operated upon cages with heating were employed to some extent, so that the temperature was maintained at about 26° C.

Operation technique.

The operations carried out for this investigation are hypophysectomy, parathyroidectomy and extirpation of the adrenals. The procedure tallies on the whole with the methods which have been described earlier (Griffith, 1942). All the operations were carried out with the animals under ether anesthesia.

The hypophysectomies were carried out on the parapharyngeal route. The animals were placed on their backs on a sheet of cork, with their heads away from the operator. The extremities were fastened down, and the head was held stretched out by passing a string fastened in the cork immediately below the front teeth in the upper jaw. The lighting was afforded by a strong, diffused light in the ceiling above the operation table and by a frontal mirror, by means of which a narrow concentrated beam could be directed on the operation area. The neck was shaved, and the skin washed with a disinfecting solution (Desivon). Subsequently a sterile cloth, which was about 20 by 20 cm. with a 3 cm. long central slit, was laid over the operation area. In the median line a $1\frac{1}{2}$ —2 cm. long incision was made in the skin over the submandibular salivary gland. This was then divided. In the first experiments tracheotomy was performed, but later as it emerged that the closing of the air passages need not necessarily be complete or was of such short duration that the animals were not injured, this procedure was abandoned. This reduced the operating time and the risk of post-operative complications. When the salivary gland tissue has been pushed aside, the trachea and larynx are seen in the median line encased in a thin layer of musculature (m. sternohyoideus). One continues down towards the base of the skull by bluntly dividing alongside the larynx and m. sternohyoideus. The large throat vessels are then lateral to the actual operation area. A number of smaller vessels sometimes branch out from the large throat vessels towards the larynx and enter the operation area. In such cases these vessels were burnt away by diathermy before the dissection was continued. If the direction is right, it is easy to get down to the base of the skull, where the long throat muscles are attached to the occipital bone on a bony ridge in the median line. This bony ridge is followed up towards the base of the skull till a ridge is reached running at right angles to it, viz. the synchondrosis between the occipital and sphenoid bones. The base of the skull is laid bare within this area, and a pair of small, slightly curved tweezers is inserted from below

towards the bone to hold the lower structures in the operation area apart, while a 2—3 mm. wide spatula delimits the latter upwards and at the same time serves as a support for the drill and prevents the latter from slipping upwards and perforating the pharynx. It is of great importance, if the operation is to be successful, that, at the place where the drilling is to be performed, the base of the skull can be easily inspected. The drilling is done with an ordinary round dental drill no. 9. The drill-hole is made in the median line, so that about $\frac{2}{3}$ of the hole lies in front of the synchondrosis. The drilling is only continued so far that the bone is just perforated. The pituitary gland is then seen at the bottom of the hole and can be sucked out with the aid of a fine glass cannula which fits into the drill hole. Sometimes the pituitary can be sucked out whole, but in the majority of instances it breaks into several pieces, and then it is important carefully to suck the edges of the hole where remains of the pituitary may be left. The whole operation can usually be performed without any great loss of blood. In any case no considerable hemorrhage need take place in connection with the drilling. During the drilling itself there is sometimes diffuse hemorrhage from the bone, and if the drill-hole is not exactly in the median line, the large vessels at the base of the skull may be injured when the drill breaks through. After the operation the animals were given some ml. of 5 % glucose solution by subcutaneous injection and put into cages heated to about 26° C. No very strict asepsis need be observed in this operation, and wound infection is rare.

In experiments designed to remove the posterior lobe, but to leave the anterior lobe, the drill-hole was made somewhat farther back than otherwise.

The completeness of the operation was established by microscopical examination of serial sections of the base of the skull within the area where the pituitary gland is situated. The parathyroids were removed with a fine pair of bent scissors, so that as little of the thyroid as possible came away with them or was injured, and if any considerable hemorrhage

ensued, it was stopped by electrocoagulation. In order to check the completeness of the operation and rule out the presence of accessory glands, the throat organs were examined microscopically in serial sections.

Extirpation of the adrenals was carried out on the dorsal route through a longitudinal incision with lateral dissection. On each side an opening one centimeter in length was made immediately below the last rib. The adrenals could then be pulled out a little with eye tweezers, and then removed with the surrounding fatty tissue by burning away the stalk.

Procedure of morphological examination.

For ordinary morphological examination the organs were fixed in 10 % formalin or Carnoy's solution. The specimens were embedded in paraffin in the ordinary manner and, in addition to alcohol, methylbenzoate was used in dehydration. The material was prepared with the microtome set for thicknesses of 5 μ or 10 μ . The sections were stained with Htx-eosin or van Gieson, the pituitary glands with Harris' hematoxylin and magdale red, or with Azan. Other granula stain which were used for the parathyroids were Mallory's phosphotungstic acid-hematoxylin, Gomori's chromic acid-hematoxylin and Azan according to Gomori. Further, in some case silver impregnation according to Gros-Schultze was used. Some parathyroids were fixed in Champy's solution and then impregnated with osmium tetroxide according to Nassonov-Kolatchev. Alkaline phosphatases were demonstrated histologically by Gomori's method.

The parathyroids of rats are of such an order of magnitude that they cannot be weighed directly with any great accuracy. Therefore I have preferred to estimate the volume of the organ instead. For this purpose the throat organs were fixed in formalin for 10 days, embedded in paraffin, and divided into serial sections 10 μ thick. The van Gieson stain was used. Using a projectoscope magnifying 170 times, the outlines of

the parathyroids in the sections were then traced and the areas enclosed planimetrically estimated. From the data collected in this way, the volume of the gland was calculated. As will be seen from the calculations given below, completely satisfactory accuracy is obtained by using every fifth section.

Table 1.

Volume determinations on 12 normal animals, 5 months.

Volume/Gm. body weight using all the sections*)	Volume/Gm. body weight using every 5th section	$\frac{x - x_{ex}}{d^2} = d/$
0.00067	0.00067	0.0000000000
0.00105	0.00105	0.0000000000
0.00087	0.00087	0.0000000000
0.00086	0.00088	0.0000000004
0.00093	0.00090	0.0000000009
0.00078	0.00078	0.0000000000
0.00099	0.00098	0.0000000001
0.00077	0.00076	0.0000000001
0.00076	0.00075	0.0000000001
0.00095	0.00094	0.0000000001
0.00106	0.00105	0.0000000001
0.00070	0.00069	0.0000000001
		$\sigma = \sqrt{\frac{d_1^2 + d_2^2 + \dots + d_n^2}{n}} =$
		$= 0.000013$

As an aid in appraising the functional state of the parathyroids, the ratio, cytoplasmic surface to nuclear surface, and the optic surface of a cross-section of the nucleus were estimated (see also the following chapter). In determining the nuclear surface, the outlines of 100 nuclei sectioned in the median plane (checked by turning the fine adjustment screw) were traced magnified 1370 times. The total surface was then planimetrically estimated, and the value so obtained — the comparison figure — used as a measure of the size of the

*) This column can be taken to give the actual values.

nucleus. The accuracy of the method emerges from the calculations presented below.

Table 2.

Optic surface of cross-sections of 100 nuclei estimated within 12 separate areas of the same parathyroid.

	36.2
	35.1
	36.3
	34.9
	36.2
	35.0
	37.2
	36.1
	35.3
	35.0
	35.2
	34.9
M =	35.6
σ =	0.718
V =	2 %

A method proposed by Chalkley (1943) was employed for calculating the relation between cytoplasm and cell nucleus. It is based on the random distribution of some points (in this case 4) among the structures in the microscopic picture. Chalkley used hairs attached to the eyepiece diaphragm; the ends of these hairs indicated the position of the points. I preferred glass-wool fibres, which could be obtained with considerably finer calibres. This method renders possible to note how many times the points coincide with certain structures in the section within a number of fields selected quite at random. Every such coincidence is called a «hit». 25 «hits» in the nucleus is taken as a unit. If e. g. the ratio, cytoplasm to nucleus, is to be determined, the number of «hits» in the cytoplasm per 25 «hits» in the nucleus is calculated, and subsequently the ratio, «cytoplasm hits» to «nucleus hits». Every such ratio is considered a unit and made the basis for the statistical evaluation. Twelve such observations, which

thus correspond to 300 »hits« in the nucleus, provide satisfactory accuracy, as will be seen from the table below. Chalkley estimated the number of »hits« at different levels of the section and thus arrived at a volume ratio. However, it proved difficult with this material to take readings at different levels in the section, and therefore the total surface has been calculated here.

Table 3.

Coefficient of variation for the cytoplasm-nucleus ratio in 7 different 5-months-old normal rats, determined in accordance with Chalkley's method.

ϵ_m	
m	
0.036	
0.052	
0.038	
0.048	
0.045	
0.058	
0.055	

The number of mitoses in the parathyroids was calculated in 20 successive 5μ sections where the diameter of the parathyroids is greatest.

Chemical analyses of blood and urine.

For the chemical analyses blood was collected by means of heart punctures with the animals under light ether anesthesia. The specimens were taken at the same time in the morning, the animals having been starved for 15 hours.

Estimations of inorganic phosphate in the plasma were carried out by Fiske and Subbarow's method (1925). About 1 ml. blood was transferred into a small centrifuge tube containing a trace of heparin. The analyses were made as soon as the specimen had been collected. 0.2 ml. plasma which did not show the least evidence of hemolysis was put into centrifuge tubes containing 2.8 ml. distilled water, and the protein was precipitated by adding 1 ml. 15 % trichloroacetic

acid. After centrifuging, to 3 ml. of the absolutely clear supernatant fluid was added 0.5 ml. of molybdate solution (15 ml. concentrated sulphuric acid + 5 Gm. sodium molybdate + distilled water to 100 ml.) and 0.3 ml. aminonaphtholsulphonic acid (0.2 Gm. 1:2:4 aminonaphtholsulphonic acid + 12 Gm. sodium metabisulphite + 2.4 Gm. sodium sulphite [crystalline] + distilled water to 100 ml.). The reaction (blue colour) was read with a Beckman spectrophotometer at 720 $m\mu$ after 15 minutes. In each series simultaneously with the specimens a standard phosphate solution containing 3 μg P/ml. was examined. An 1 cm. cuvette was employed.

An absorption curve for the blue phosphorus molybdenum complex was constructed. The spectral band in question was 1.54 $m\mu$ wide at 560 $m\mu$ and 0.96 $m\mu$ at 800 $m\mu$. The mean of the specific extinction coefficient K emerges from the table below.

Table 4.

Wave length $m\mu$	580	600	620	640	660	680	700	720	740	760	780	800
K	81	88	97	103	106	108	110	111	111	110	110	110

Further, the blue colour was followed by means of a time graph (Fig. 1).

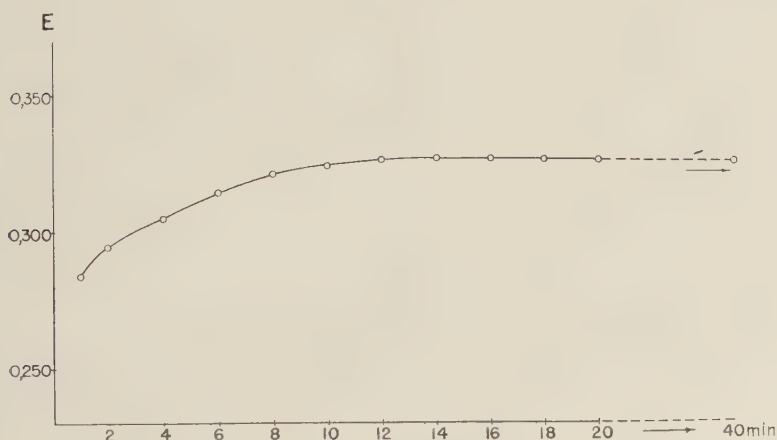


FIG. 1. Time curve for colour reaction in the determination of phosphorus. 3 μg P/ml. Breadth of band 0.99 $m\mu$.

A standard comparison graph with specimens in rising concentrations was constructed (Fig. 2).

For the methodical error see following table.

Table 5.

3 μg P/ml.	K
	107
	105
	104
	105
	106
	107
	107
	107
	108
	108
	110
	109
	110
	108
	110
	111
	112
	111
	110
	108
	112
	110
	111
	112
	112
	112
	112
M =	109
σ =	2.41
V =	2 %

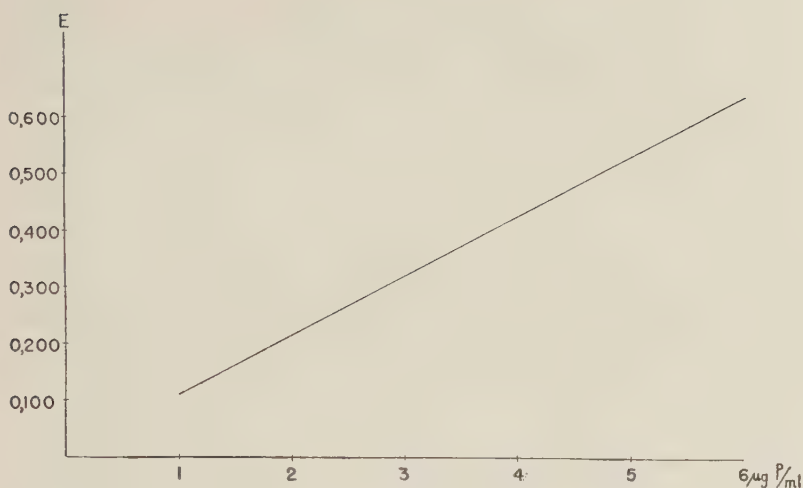


FIG. 2. Standard comparison curve for 1—6 $\mu\text{g P/ml}$.

Blood calcium estimations were made according to Clark and Collip using 1 ml. plasma, calcium being precipitated in the form of oxalate and the calcium oxalate being dissolved in sulphuric acid, whereby the oxalic acid is released, and is then oxidised quantitatively with potassium permanganate. Double tests were made. 5 to 6 ml. blood being required, this calcium estimation necessitate the killing of the animals.

Simultaneously with the blood calcium analyses, serum protein estimations were carried out by means of nitrogen determination using Kjeldahl's micromethod with the Kirsten apparatus. 0.2 ml. plasma was used for each determination, and double tests were made. The nonprotein nitrogen was supposed to be stable, and it was not considered necessary to make any corrections for it.

The phosphate in the urine was estimated with uranyl acetate, which in the presence of a trace of acetic acid gives a yellowish-white flocculent precipitate (see G. Hammarsten, Thesis, 1937).

Statistical symbols.

The following statistical symbols and formulas have been employed.

M = the arithmetical mean

$$\sigma = \sqrt{\frac{a^2}{n-1}}$$

ϵ_m = the standard error of the mean = $\sqrt{\frac{a^2}{n/n-1}}$ where a^2

= the sum of squares of the differences from the arithmetical mean and n = the number of observations.

$$t = \sqrt{\frac{n_1 n_2 / n_1 + n_2 - 2}{n_1 + n_2}} \frac{\bar{x} - \bar{y}}{\sqrt{n_1 S_1^2 + n_2 S_2^2}}$$

$\bar{x}$ = mean value of series 1

$\bar{y}$ = » » » » 2

$$n_1 S_1^2 = x_1^2 + x_2^2 + \dots + x_{n_1}^2 - n_1 \bar{x}^2$$

$$n_2 S_2^2 = y_1^2 + y_2^2 + \dots + y_{n_2}^2 - n_2 \bar{y}^2$$

t is distributed in Student's distribution with $n_1 + n_2 - 2$ degrees of freedom.

$$\text{Coefficient of variation } V = 100 \frac{\sigma}{M} \%$$

The different levels of significance are denoted by stars, according to the system described in Bonnier-Tedin (1940).

Chapter 4.

MORPHOLOGICAL METHODS OF APPRAISING THE FUNCTIONAL STATE OF THE PARATHYROIDS.

Perhaps the most usual method of demonstrating hyperactivity or hypoactivity of endocrinous glands is to establish hyperplasia or hypoplasia of the organ. In respect of the parathyroids such attempts have been made, *inter alia*, with animals treated with pituitary extract and hypophysectomized animals respectively. This method, however, is impaired by a number of drawbacks, the normal size variations of the parathyroids being considerable; and therefore a large number of observations are necessary to obtain valid differences. The method will, probably with reason, be considered rather inaccurate, as there is some evidence to show that the parathyroids have a considerable functional reserve. Thus if the organ is stimulated, it may be expected in the first place to increase its function and possibly to undergo hypertrophy at a later stage. Hence, the stimulus must be strong and act for a considerable period if it is to be possible to establish hyperactivity of the organ by demonstrating hypertrophy.

In this investigation various approaches were explored to arrive at a more sensitive criterion of a changed parathyroidal function than observations as to the size of the organ might be expected to supply.

The phosphatase enzyme system is of the greatest importance for the metabolism in the cell. The presence of certain phosphatases in the tissues can be demonstrated by histological methods. In the adrenal glands, the testes and the

thyroid, a well-marked change takes place in the distribution, and a considerable reduction of the amount, of alkaline phosphatase after hypophysectomy, which to a large extent depresses the function of the organs mentioned. Alkaline phosphatases might be supposed to behave in a similar manner in the parathyroids (Dempsey and his associates 1942). On the other hand, after hypophysectomy in the parathyroids no corresponding reduction or change in the distribution of alkaline phosphatase could be definitely established, nor did implantation of pituitary material lead to any definite changes in this respect to be demonstrated by Gomori's method.

As has already been suggested in the introduction, recent cytological advances have directed attention — thanks to the contributions of Caspersson and his associates — to the importance of the nucleus for the formation of protein in the cell. In a paper dealing with size variations of the nuclei in the liver at different phases of the liver rhythm, Caspersson and Holmgren (1934) reported that the size of the nuclei culminates when the liver glycogen reaches its highest value. Lagerstedt (1949) has shown that the size of the nuclei in the liver decreases during starvation, as the synthesis of protein is reduced, and increases if the starving animal is supplied with ample protein. It emerges also from Lagerstedt's investigations that the ratio, cytoplasm to nucleus, is affected by different functional states of the cell. Thus, in starving animals this ratio is apt to drop in the liver. The size of the nucleoli also shows a characteristic change. They decrease in size in starving animals and increase in animals on a diet abundant in protein.

In order to obtain information as to how far these data may be of value for appraising the functional state of the parathyroids, the organ was examined under different conditions. The size of the nuclei was recorded in the manner described in the chapter on materials and methods and further, the ratio, cytoplasm to nucleus, was determined by Chalkley's method. At the outset the intention was to investigate also the size variations of the nucleoli, but it proved

difficult to identify them with certainty in the case of normal or reduced functional states of the parathyroids, and therefore this detail had to be disregarded. Chalkley's procedure for determining the ratio, cytoplasm to nucleus, described in the foregoing, presents several advantages over the usual ones. Thus it is entirely independent of the shape of the structures which are to be measured, and, further, the values obtained can be analysed statistically for each object measured. The measurements are also made direct on the section.

Animals reared on a diet abundant in phosphorus and poor in calcium, develop an increased phosphorus content and a decreased calcium content of the body fluids, and this change in the concentration of these substances entails hyperactivity of the parathyroids and hypertrophy of the organ. Thus, investigations of the ratio, cytoplasm to nucleus, and of the sizes of the nuclei in such animals may afford a conception of whether these findings are changed in the case of an increased function of the organ, and also of in what manner they are changed. In these investigations 4-month-old male rats were placed on a diet abundant in phosphorus for a period of 1 month. The calcium-phosphorus ratio in the diet was 1:8, with an actual calcium content of 0.2 %

On the other hand, with a diet abundant in calcium and poor in phosphorus, an increased calcium content and a decreased phosphorus content of the blood are found, and in consequence a reduced function and hypertrophy of the parathyroids. Investigations, corresponding to those carried out with diets abundant in phosphorus, were performed with diets abundant in calcium which had a calcium-phosphorus ratio of 8:1 with an actual calcium content of 2.0 %.

Another method of producing hypo-activity is administration of the parathyroid hormone or a substance with the same effect. A series of animals were given 0.3 ml. of AT 10 daily for 15 days.

The cytological changes in the parathyroids recorded in experiments with, first, a diet abundant in phosphorus,

second, a diet abundant in calcium, and third, AT 10 administration are presented in the following table.

Table 6.

Behaviour of nuclei and cytoplasm in the parathyroids at different functional states of the organ. 5-month-old rats.

	No of animals	Cytoplasm Nucleus $\pm \varepsilon_m$	Nuclear surface*)	Cytoplasmic surface*) (calculated)	Mitoses
Normal	7	M = 3.17 ± 0.058	M = 40.1	M = 126.9	0—2
Diet abundant in phosphorus	5	2.33 ± 0.14	44.1	102.8	12
		2.65 ± 0.09	52.5	139.1	23
		2.22 ± 0.12	49.7	110.3	18
		2.40 ± 0.15	44.0	105.6	21
		2.53 ± 0.10	51.9	131.3	30
		M = 2.43 ± 0.056	M = 48.4	M = 117.8	
		t = 8.9***			
		d. f. = 142			
Diet abundant in calcium	5	2.06 ± 0.12	31.6	65.1	0
		2.69 ± 0.12	31.7	85.3	0
		2.25 ± 0.08	37.2	83.7	0
		2.30 ± 0.07	32.0	73.6	0
		2.21 ± 0.10	37.0	81.8	0
		M = 2.30 ± 0.051	M = 33.9	M = 77.9	
		t = 10.7***			
		d. f. = 142			
AT10	6	2.65 ± 0.10	32.0	84.8	0
		2.30 ± 0.16	31.9	73.4	0
		2.54 ± 0.11	27.3	69.3	0
		2.28 ± 0.11	31.5	71.8	0
		2.71 ± 0.06	36.9	100.0	0
		2.59 ± 0.14	35.8	92.7	0
		M = 2.51 ± 0.050	M = 32.6	M = 82.0	
		t = 8.4***			
		d. f. = 154			

*) Comparison figures, see also *Materials and Methods*, p. 42.

As will be seen from the data in the table, the ratio, cytoplasm to nucleus, changes with different functional states of the parathyroids. Hypo-activity (AT 10 and diet abundant in calcium) is associated with a ratio markedly lower than in normal animals. The nuclear surface is also obviously smaller than normally, however, and this applies also to the cytoplasm, which thus, relatively speaking, has decreased more in quantity than has the nucleus. In hyperactivity, there is also a decrease in the ratio, cytoplasm to nucleus, but in this case the nuclear surface is more or less increased, and the cytoplasm is normal or sometimes somewhat enlarged, but to a lesser degree than the nucleus. Thus, from what has been said above, it appears that these calculations provide a possibility of demonstrating morphologically an increased or decreased function of the parathyroids.

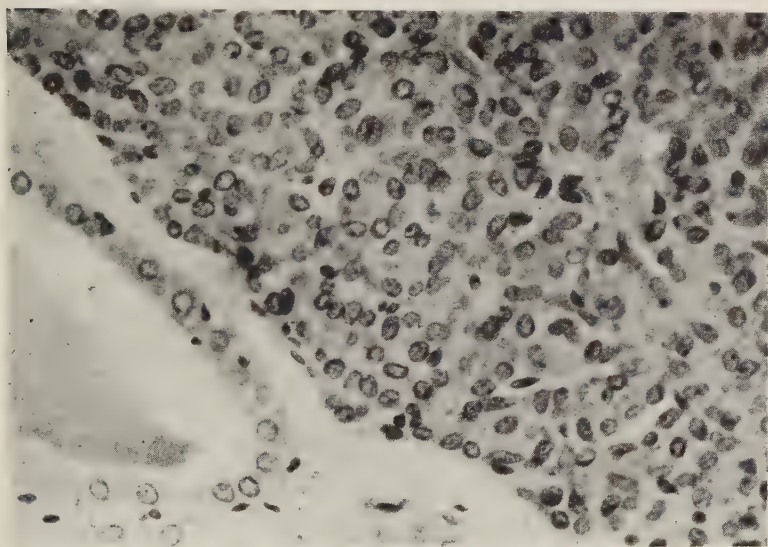


FIG. 3. Parathyroids and adjacent parts of the thyroid (5-month-old normal rat). $\times 500$.

It is naturally important to ascertain whether the cytological changes in the parathyroids described here can be

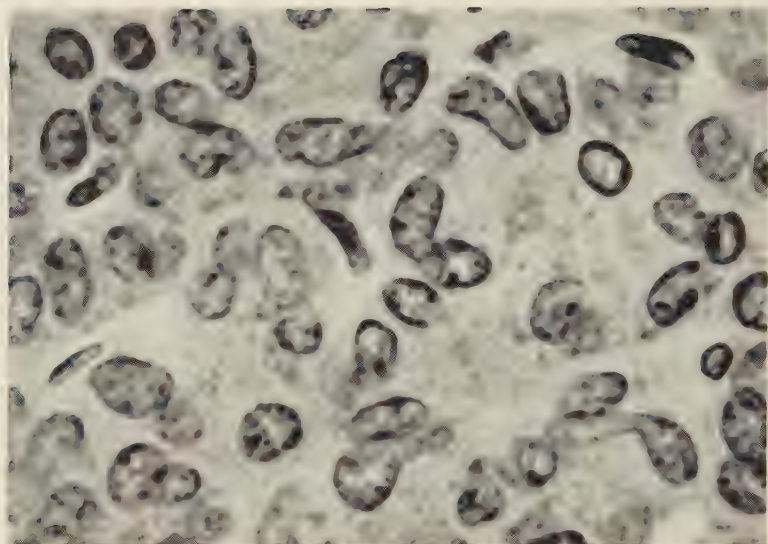


FIG. 4. Parathyroids (5-month-old normal rat). $\times 1350$.

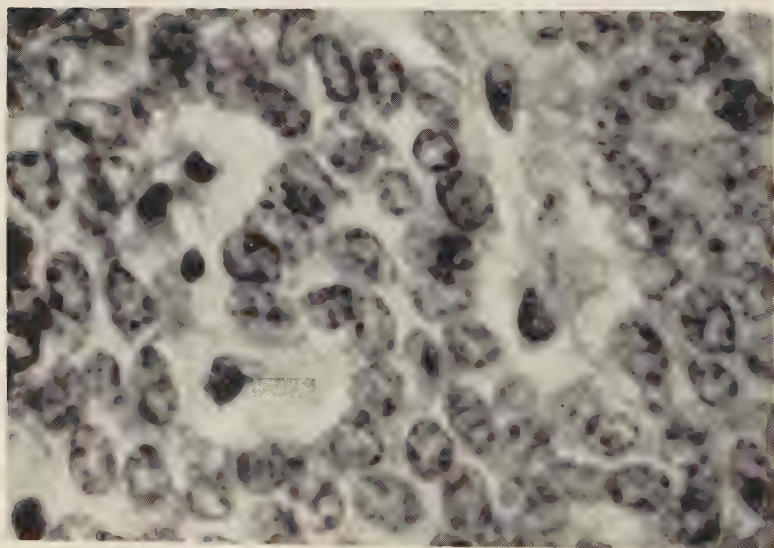


FIG. 5. Parathyroids, 5-month-old rat for 15 days on a diet abundant in calcium. $\times 1350$.

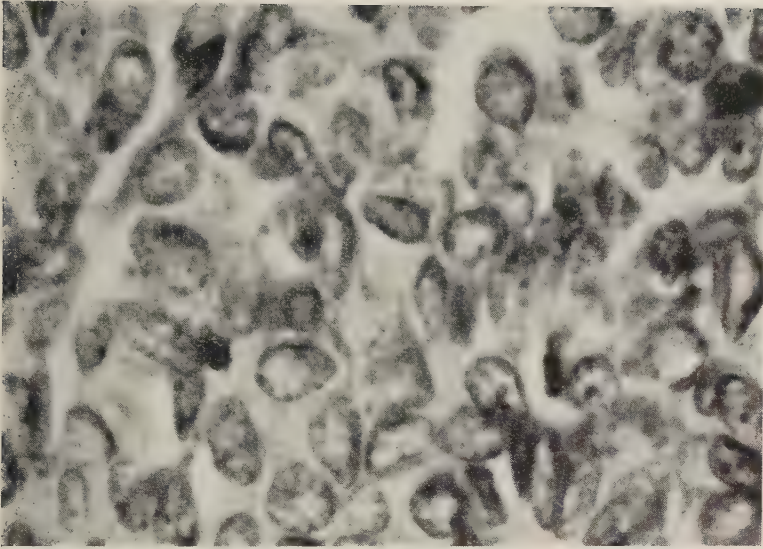


FIG. 6. Parathyroids, 5-month-old rat for 15 days on a diet abundant in phosphorus. $\times 1350$.

demonstrated shortly after a functional change, and whether they develop already before any hypertrophy of the organ is observed.

In order to investigate this point, a group of animals were placed on a diet abundant in phosphorus and poor in calcium, and killed after 2, 5, 10 and 15 days respectively. The blood phosphorus was checked at the time of death and was found to be increased in the animals killed 5, 10 and 15 days after the diet had been instituted, while those which were killed on the second day did not show any definite divergencies. The parathyroids of the animals were examined in respect of the ratio, cytoplasm to nucleus, and the volume of the organ was also calculated (see the tables).

It emerges from the tables that as early as after 5 days the ratio, cytoplasm to nucleus, presents values which definitely indicate hyperactivity, while, on the other hand, not until after 10 days does the volume show a practically, and

after 15 days, definitely, established increase. Hence the present investigations show that by the cytological method employed here functional changes in the parathyroids can be

Table 7.
Volumes of the parathyroids.

	Normal controls cu. mm./Gm	5 days on diet abundant in phosphorus cu. mm./Gm	10 days on diet abundant in phosphorus cu. mm./Gm	15 days on diet abundant in phosphorus cu. mm./Gm
No. of animals	12	12	14	12
	0.00074	0.00119	0.00124	0.00149
	0.00111	0.00148	0.00177	0.00286
	0.00121	0.00116	0.00112	0.00128
	0.00179	0.00127	0.00137	0.00175
	0.00097	0.00175	0.00188	0.00189
	0.00089	0.00120	0.00195	0.00164
	0.00115	0.00182	0.00154	0.00153
	0.00123	0.00117	0.00130	0.00172
	0.00116	0.00109	0.00176	0.00220
	0.00100	0.00136	0.00162	0.00200
	0.00120	0.00182	0.00153	0.00174
	0.00172	0.00196	0.00149	0.00200
			0.00131	
			0.00177	
	M = 0.00118	M = 0.00144	M = 0.00155	M = 0.00184
	σ = 0.00031	σ = 0.00031	σ = 0.00023	σ = 0.00042
	ϵ_m = 0.000089	ϵ_m = 0.000089	ϵ_m = 0.000062	ϵ_m = 0.000120
		t = 2.057	t = 3.484**	t = 4.412***
		d. f. = 22	d. f. = 24	d. f. = 22

demonstrated much earlier than is possible by the use of volume determinations. Further, under suitable conditions it is possible to draw definite conclusions from cytological examinations of a single specimen as to the functional state of the organ concerned, while a size determination of the organ most frequently calls for a large number of animals if a definite result is to be arrived at.

Table 8.

Behaviour of nuclei and cytoplasm in the parathyroids of rats on a diet abundant in phosphorus for:

	No. of animals	Cytoplasm Nucleus	$\pm \epsilon_m$	Nuclear surface ^{*)}	Cytoplasmic surface ^{*)} (calculated)	Mi- toses
Normal	7	M =	3.17 ± 0.058	M = 40.1	M = 126.9	0—2
2 days	5		2.92 ± 0.12	41.7	121.8	1
			3.14 ± 0.16	42.3	132.8	3
			3.33 ± 0.15	41.2	137.2	0
			3.14 ± 0.16	40.1	125.9	0
			2.94 ± 0.10	40.0	117.6	1
		M =	3.09 ± 0.064	M = 41.1	M = 127.1	
		t =	0.8			
		d. f. =	142			
5 days	6		2.55 ± 0.13	50.1	127.8	18
			2.48 ± 0.08	47.3	117.3	11
			2.47 ± 0.14	48.2	119.1	22
			2.62 ± 0.13	41.0	107.4	8
			2.60 ± 0.13	46.4	120.6	14
			2.61 ± 0.08	46.5	121.4	16
		M =	2.56 ± 0.047	M = 46.6	M = 118.9	
		t =	8.0***			
		d. f. =	154			
10 days	6		2.45 ± 0.11	42.0	102.9	14
			2.46 ± 0.07	51.3	126.2	29
			2.60 ± 0.18	45.8	123.7	19
			2.52 ± 0.15	51.0	128.5	16
			2.51 ± 0.10	47.2	118.5	22
			2.62 ± 0.16	50.0	131.0	27
		M =	2.53 ± 0.053	M = 47.0	M = 121.8	
		t =	8.0***			
		d. f. =	154			
15 days	7		1.81 ± 0.12	46.4	84.0	15
			2.15 ± 0.15	50.1	107.7	23
			2.04 ± 0.10	47.0	95.9	18
			2.11 ± 0.07	50.0	105.5	41
			2.29 ± 0.13	56.2	128.7	48
			2.47 ± 0.15	48.2	119.1	21
			2.33 ± 0.12	49.9	116.3	27
		M =	2.19 ± 0.050	M = 49.7	M = 108.2	
		t =	13.0***			
		d. f. =	166			

*) Comparison figures, see also *Materials and Methods*, p. 42.

Chapter 5.

THE ANATOMY AND HISTOLOGY OF THE PARATHYROIDS AND CERTAIN CHEMICAL FEATURES OF THE BLOOD AND URINE IN NORMAL RATS.

In mammals the parathyroids are derived from the 3rd and 4th branchial clefts, and normally the fully developed gland is situated in or close to the thyroid. In the majority of mammals there are two glands on either side of the throat, but in normal rats there is only one. The anatomy and histology of the parathyroids have been investigated by a number of workers from the time of Sandström (1880).

The anatomy and histology of the parathyroids in albino rats have been studied by, *inter alios*, Jackson (1916) and Hoskins (1924), who showed that only one parathyroid is found on each side in these animals and is situated in the lateral upper edge of the thyroid. Hoskins and Chandler (1925), using serial sections, examined the throat organs right down to the mediastinum and could only find accessory parathyroids in 5 cases out of 39, and then only one accessory gland in each case. Other workers, e. g. Erdheim (1914), state that accessory parathyroids occur fairly frequently.

The volume of the parathyroids in rats of different ages has been studied by Blumenfeld and Rice (1938). Campbell and Turner (1942) investigated the weight of the parathyroids in adult rats and found that female rats have larger para-

thyroids in relation to their body weight. Sinclair (1941) has shown that young rats have proportionally larger parathyroids, the parathyroids being about equally large in unmated female rats and in males.

Hoskins (1924) describes three kinds of cells in the parathyroids of rats, viz. the chief cells without granular cytoplasm, which may fill the whole gland, and further, light-coloured cells with more bulky cytoplasm, which occur mostly in the superficial layers and probably are a modification of the chief cells. There are also chromophilous cells which are smaller than those described above, have dark nuclei, and are situated in a thin zone near the margin of the gland. The author states that the chief cells are those which are functionally important, and that the chromophilous cells are merely the result of postmortal changes. Anselmino and Hoffman (1933) have observed the same type of cells in the rats examined by them. Rucart (1949) and a number of other workers were only able to identify one kind of cell in rats — the chief cells. Rosof (1934) carried out a histological examination of rats of different ages and found that the number of mitoses in the parathyroids diminished with age, and that the size of the cells decreased after the onset of sexual maturity. This author demonstrated an osmiophilic substance in the cytoplasm and found that dark osmiophilic cells were secretory. Such cells were found in 5—25 % of the glands.

In my material I found the parathyroid glands fairly regularly situated lateral in superficial portions of the thyroid lobes. In a few instances one gland was found within the musculature adjacent to the thyroid, and in occasional cases farther dorsal alongside of or even behind the trachea. Among approximately 300 throat organs examined in serial sections the parathyroid gland was absent on one side in two instances. Five times, in addition to the ordinary glands, a further one was found on one side, viz. situated atypically. In no case was more than one accessory gland met with.

The parathyroids are encased in a rather delicate capsule of connective tissue, which becomes coarser with age. The

parenchyma is divided into smaller aggregations of cells by fine strands of connective tissue and capillaries. The parenchyma cells usually lie in large agglomerations without any recognizable order. Very occasionally, however, a trabecular or pseudo-alveolar arrangement is observed. These latter structures are more marked in material fixed with formalin than in that fixed with Carnoy's solution. No acini or follicles were noted. In the cases where the parathyroids lay in the surface of the specimen, a narrow zone was often seen within the superficial strata, where the cells were smaller and darker than otherwise. This change is obviously a result of shrivelling during fixation.

The parenchyma of the parathyroids is built up of a single cell type with a cytoplasm which often cannot be delimited with certainty from adjacent cells. The cytoplasm exhibits a certain but inconsiderable density and sometimes just a trace of granulation. These details are easily visible in material fixed with Carnoy's solution, while the cytoplasmic areas in formalin-fixed preparations appear empty for the most part. The appearance of the nucleus varies somewhat, but it is mostly round or elliptical. Occasional cells have oblong nuclei, however, and irregular outlines are sometimes seen. The structure of the nucleus is somewhat loose with a fine chromatin network. One or more not sharply delimited nucleoli are often noted. In new-born and young animals mitoses are fairly common, whereas they are scanty in older animals.

Most of the ordinary granula stains were applied to the parathyroids but failed to disclose granula in the cytoplasm. Various modifications of silver impregnation procedures also gave negative results. Alkaline phosphatase was met with in the parathyroids, lying in connective tissue septa, particularly round the capillaries, and also within the nuclei of the parenchyma cells.

The volume of the parathyroids was estimated for rats of different ages. Thus volume determinations were performed with male rats on a normal diet in the age groups, new-born,

50 days, 65 days, 80—90 days, and 150—200 days. The separate values are given in the Tables I—V in the appendix, where, firstly, the absolute volume is stated, and secondly, the volume per Gm. body weight. The corresponding values for adrenal glands, testes and kidneys in mg. have been included in the tables for purposes of comparison. The following table contains the means of the determinations pertaining to the parathyroids.

Table 9.
Normal rats.

	No.	Parathyr. volume in cu. mm.	Parathyr. volume per Gm. body weight
		$M \pm \epsilon_m$	$M \pm \epsilon_m$
New-born	19	—	0.00230 ± 0.00004
50 days	11	0.0958 ± 0.0065	0.00170 ± 0.000132
65 days	11	0.1135 ± 0.0069	0.00112 ± 0.000070
80—90 days	14	0.1317 ± 0.0072	0.00089 ± 0.000044
150—200 days	16	0.1364 ± 0.0083	0.00067 ± 0.000046

The ratio, cytoplasmic to nuclear surface was calculated according to Chalkley's method, and a measure of the nuclear surface was obtained by calculating the surface of 100 nuclei. The corresponding value for the cytoplasm surface was subsequently calculated. These details were investigated with new-born, 30-day, 60-day and 150-day rats, and the results will be seen from the table below.

Table 10.
Details of nuclei and cytoplasm in the parathyroids of normal rats
at different ages.

	No. of animals	Cytoplasm Nucleus $\pm \epsilon_m$	Nuclear surface*)	Cytoplasmic surface*) (calculated)	Mi- to- ses
New-born	5	3.34 ± 0.17	33.2	110.9	
		2.99 ± 0.11	37.7	112.7	
		3.09 ± 0.15	39.1	120.8	
		3.10 ± 0.10	38.9	120.6	
		2.96 ± 0.11	35.6	105.4	
		$M = 3.10 \pm 0.059$	$M = 36.9$	$M = 114.1$	

	No. of animals	Cytoplasm Nucleus $\pm \epsilon_m$	Nuclear surface*)	Cytoplasmic surface*) (calculated)	Mi- to- ses
1 month	5	3.08 $\pm$ 0.13	35.7	110.0	
		3.04 $\pm$ 0.22	32.5	98.8	
		2.94 $\pm$ 0.11	32.8	96.4	
		3.57 $\pm$ 0.17	37.5	139.9	
		3.48 $\pm$ 0.19	37.5	130.5	
		M = 3.22 $\pm$ 0.080	M = 35.2	M = 113.9	
2 months	5	3.14 $\pm$ 0.06	35.8	112.4	
		3.55 $\pm$ 0.19	37.8	134.2	
		3.53 $\pm$ 0.14	38.0	134.1	
		3.05 $\pm$ 0.14	38.3	116.8	
		2.90 $\pm$ 0.14	38.1	110.5	
		M = 3.23 $\pm$ 0.070	M = 37.6	M = 121.6	
5 months	7	3.06 $\pm$ 0.11	39.2	120.0	2
		3.05 $\pm$ 0.16	40.4	123.2	0
		3.41 $\pm$ 0.13	38.5	131.3	1
		2.94 $\pm$ 0.14	41.0	120.5	2
		3.36 $\pm$ 0.15	39.9	134.1	0
		3.26 $\pm$ 0.19	39.1	127.5	0
		3.08 $\pm$ 0.17	42.8	131.8	1
		M = 3.17 $\pm$ 0.058	M = 40.1	M = 126.9	

*) Comparison figure, see further *Materials and Methods*, p. 42.

Blood-chemical investigations were carried out parallel with the morphological studies, and the normal rate for inorganic phosphorus in the plasma were determined.

	Number	M $\pm \epsilon_m$ mg.0/o
Rats ♂ about 5 months old, normal diet	30	5.8 $\pm$ 0.09
- - 2 - - - -	31	7.5 $\pm$ 0.18

Young rats have a somewhat higher blood phosphorus than older ones; from and including 3—4 months the rate is stable.

In young rats the normal variations in the blood phosphorus rate are also considerably larger.

The blood calcium analyses gave the following result:

	Number	$M \pm \epsilon_m$ mg. %
Rats ♂ about 5 months old, normal diet	33	11.3 ± 0.12

The following rates were noted in plasma protein analyses on normal animals about 5-months-old:

	Number	$M \pm \epsilon_m$ %
	14	6.4 ± 0.14

The amount of urine excreted per 24 hours was recorded, and for normal animals the following amount were obtained

	Number	$M \pm \epsilon_m$ ml.
	20	2.9 ± 0.35

The amount of phosphate phosphorus in the urine in these cases was:

	Number	$M \pm \epsilon_m$ mg.
	20	4.6 ± 0.50

Discussion.

In respect of the anatomical features, the results tally perfectly well with those of earlier investigators. The occurrence of accessory parathyroids in my material is somewhat sparse, the incidence agreeing better with the findings of Hoskins than with those of Erdheim. The variations may naturally be due to the differences displayed by separate rat strains.

Having been unable to identify the different types of cells in the parathyroids of rats which have been described by a number of authors (*inter alios* Hoskins, Anselmino and his associates), I am of the opinion that there is only one kind

of cell, i.e. the so-called chief cells. This conception tallies with the observations of Rucart and others. What appear to be somewhat varying types of cells in the parathyroids may actually be artefacts. For example, darker and more shrivelled cells are often seen towards the periphery of the gland in cases where the parathyroids have been lying on the surface of the specimen, but no such pictures are observed when the gland is entirely embedded in another tissue. Here we are certainly confronted with a fixation effect, viz. more shrivelling in superficial parts. Nor has the more or less pronounced pseudo-alveolar structure, which is sometimes stated to be the normal picture, been observed to any great extent. In Carnoy-fixed material at least, the cells rather appear to be quite irregularly scattered.

As will have emerged from the tables, the absolute size of the parathyroids increases with age, but between 80—90 and 150—200 days the increase is very slight. Thus it appears as though the parathyroids grow until the rats are about 3 months old, and that subsequently the volume remains stable. The relative size of the parathyroids, i.e. the size per Gm. body weight, also changes, i.e. the gland is largest in the new-born and then decreases in size through all the age-groups.

The study of the ratio, cytoplasm to nucleus, and the calculations of the nuclear and cytoplasmic surfaces showed that, on the whole, this ratio is stable in the separate age-groups investigated. In these normal animals the ratio, cytoplasm to nucleus, displays but small variations, the figure being near to or just above 3. Nor does the nuclear surface exhibit any considerable differences as between the different age groups. Thus it follows that on the whole the cytoplasmic surface is stable for different ages. In the oldest group, however, there seems to be a certain tendency towards larger nuclear, and thus also towards larger cytoplasmic surfaces.

Chapter 6.

THE PARATHYROIDS AND THE BLOOD CHEMISTRY DURING DIFFERENT FUNCTIONAL STATE OF THE PITUITARY GLAND

1. *The effect of an excess of anterior pituitary hormone.*

In order to study the behaviour of the parathyroids in the presence of an excess of anterior pituitary hormone, fresh anterior lobe of calf pituitary was subcutaneously administered to rats. The material was aseptically collected, the anterior lobe isolated and ground to a practically homogeneous pulp, which was injected every day for 10 days to a total of about 4 Gm. Two groups of rats were treated in this manner, one group aged 80—90 days, and the other aged 150—200 days. The volume of the parathyroids was subsequently calculated, and in some case the ratio, cytoplasm to nucleus, was recorded (see below).

The results of the volume determinations in the individual cases will be seen from Tables VI and VII in the Appendix, where also particulars of the adrenals and testes are given. The figures should be compared with the corresponding values for the normal material in Chapter 5.

The blood phosphorus was also examined in 150—200-day-old rats 10 days after the pituitary treatment had been instituted, the following rate being noted:

No. of animals	$M \pm \epsilon_m$ mg. %
11	8.0 ± 0.3
(Normal rate 5.8 ± 0.09 mg. %)	

Table 11.

Behaviour of nuclei and cytoplasm in the parathyroids of 5-month-old rats after pituitary administration (killed after 10 days) and after hypophysectomy (killed after 20 days).

	No. of animals	Cytoplasm Nucleus $\pm \epsilon_m$	Nuclear surface*)	Cytoplasmic surface*) (calculated)	Mitoses
Normal	7	3.17 ± 0.058	$M = 40.1$	$M = 126.9$	0—2
Pituitary administration	5	2.56 ± 0.18	47.0	125.0	8
		2.41 ± 0.17	41.6	100.3	3
		2.49 ± 0.12	40.7	101.3	4
		2.45 ± 0.16	43.5	106.6	7
		2.60 ± 0.17	44.2	114.9	5
		$M = 2.50 \pm 0.070$	$M = 43.4$	$M = 109.6$	
		$t = 7.3^{***}$			
		d. f. = 142			
Hypophysectomy	6	2.18 ± 0.13	34.1	74.3	0
		2.03 ± 0.14	29.2	59.3	0
		1.50 ± 0.07	26.9	40.4	0
		1.51 ± 0.08	32.8	49.5	0
		2.54 ± 0.16	23.0	58.4	0
		2.08 ± 0.09	28.4	59.1	0
		$M = 1.97 \pm 0.064$	$M = 29.1$	$M = 56.8$	
		$t = 13.9^{***}$			
		d. f. = 154			

*) Comparison figures, see further *Materials and Methods*, p. 42.

A group of rats which had been given 20 units growth hormone (phyol) from the anterior lobe of the pituitary gland, were also examined in a similar manner (see Table VIII in the Appendix and below). The phosphorus rate for this group was:

No. of animals	$M \pm \epsilon_m$ mg. %
11	7.6 ± 0.4

The blood calcium rate in the rats treated with growth hormone was 10.6 ± 0.24 mg. % (normal, 11.3 ± 0.12 mg. %). This rate discloses no definite divergency from normal.

Table 12.

Means pertaining to the size of the parathyroids.

	No.	Parathyr. volume in cu. mm. $M \pm \epsilon_m$	Parathyr. volume per Gm. body weight $M \pm \epsilon_m$
Animals treated with pituitary gland			
80—90 days old	16	0.1720 ± 0.0099	0.00113 ± 0.000050
Animals treated with pituitary gland			
150—200 days old	16	0.2194 ± 0.0192	0.00107 ± 0.000094
Animals treated with growth hor- mone			
150—200 days old	11	0.1580 ± 0.0086	0.00083 ± 0.000044
Normal animals			
80—90 days old	14	0.1317 ± 0.0072	0.00089 ± 0.000044
Normal animals			
150—200 days old	16	0.1364 ± 0.0083	0.00067 ± 0.000046

As to the volume determinations, it emerges from the tables that in both groups the pituitary-treated animals exhibit a definite enlargement of the parathyroids. The animals which had received growth hormone also show a somewhat larger mean volume than the normal. A more detailed statistical analysis of the results of pituitary treatment gives a *t*-value of 3.47** (d. f. = 28) for 80—90-day rats. For 150—200-day rats the *t*-value is 3.74*** (d. f. = 30), and for the rats treated with growth hormone it is 2.30* (d. f. = 25). The ratio, cytoplasm to nucleus, in the parathyroids of the animals treated with pituitary gland is clearly decreased, and the nuclear surface appears to be increased. Apart from the fact that the nuclei appear to be larger than in normal rats, they also present a somewhat different structure. They display a looser and finer chromatin network as well as large and well-marked nucleoli. The cytoplasmic surface shows definite divergencies

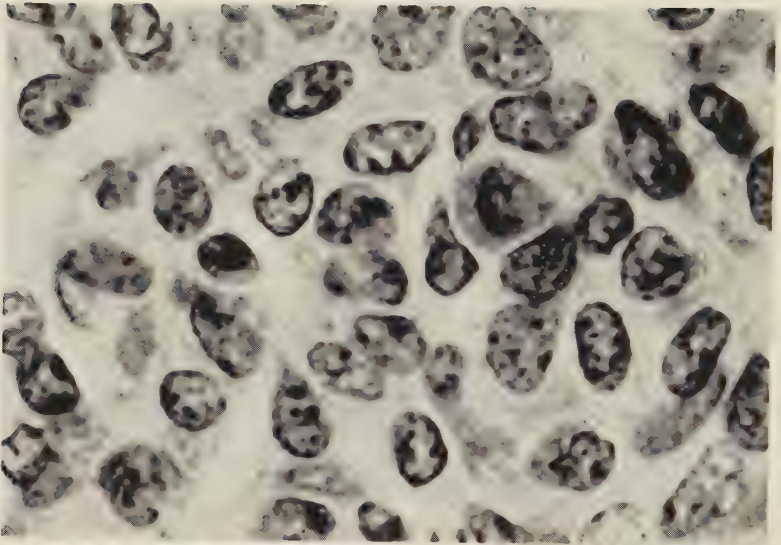


FIG. 7. Parathyroids, 5-month-old pituitary-treated rat. $\times 1350$.

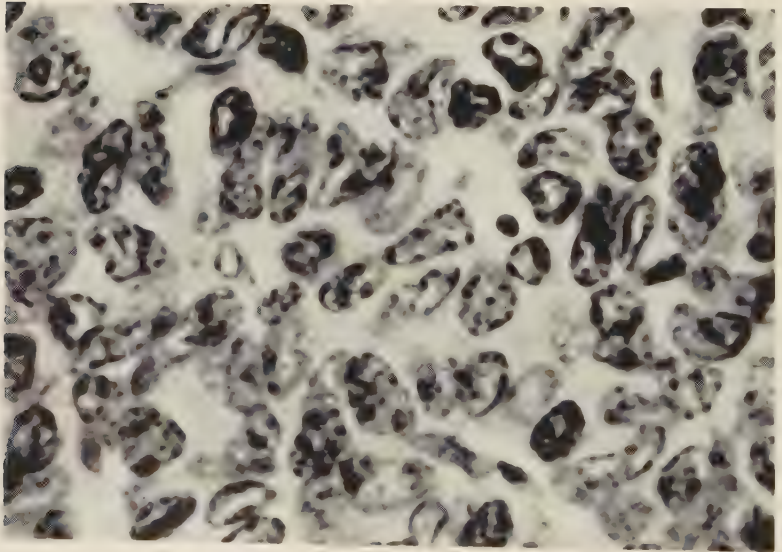


FIG. 8. Parathyroids, 5-month-old hypophysectomized rat (21 days after the operation). $\times 1350$.

from normal. An increased number of cells undergoing mitosis were also observed. Thus there are morphological changes which tally with those found associated with parathyroidal overactivity.

2. *The effects of hypophysectomy.*

The parathyroids were also examined following hypophysectomy. The size of the organ was determined, and the figures are given in Table IX in the Appendix, where the changes in weight of the animals and details of the testes and adrenals are also recorded.

Table 43.

Means pertaining to the size of the parathyroids in hypophysectomized 150—200-day-old male rats.

No.	Parathyr. volume in cu. mm.	Parathyr. volume per Gm. body weight at op.	Parathyr. volume per Gm. body weight at death
		$M \pm \epsilon_m$	$M \pm \epsilon_m$
11	0.1199 ± 0.0083	0.00054 ± 0.000037	0.00081 ± 0.000056

The absolute volume of the parathyroids of the hypophysectomized rats proved to be somewhat less than that of normal animals of the same age, but the difference is not statistically valid. The hypophysectomized animals decrease fairly considerably in weight after the operation, and if the volume of the parathyroids per Gm. body weight is correlated with the body weight at death, it is found that relatively the organ is somewhat larger than in the normal animals. Hence the parathyroids obviously lose less in size than would correspond to the general loss of body weight. The corresponding figures for testes and adrenals, where there is a statistically valid decrease in the organs with a wide margin of accuracy, are sharply contrasted with these data. This applies both to the absolute value and the value per Gm. body weight. Investigation of the ratio, cytoplasm to nucleus, in the parathyroids

(see Table p. 66) has shown that the hypophysectomized animals have a markedly lowered ratio. The nuclear surface is also clearly reduced, as is the cytoplasmic surface, which thus, comparatively speaking, exhibits a considerably greater decrease than the nuclear surface. The cell picture in the parathyroids after hypophysectomy is also changed in some respects. The nuclei become small and more irregular in shape; they further become darker, often pyknotic, and no mitoses are to be found.

For comparison of the changes see photomicrographs of the parathyroids in pituitary-treated, normal and hypophysectomized animals respectively (Figs. 4, 7 and 8).

In addition to the morphological investigations, a number of chemical data of blood and urine in the hypophysectomized rats were also recorded. The blood phosphorus in 5-month-old hypophysectomized animals was estimated 15—25 days after the operation.

No. of animals	$M \pm \epsilon_m$ mg. %
20	4.6 ± 0.1
(Normal rate, 5.8 ± 0.09 mg.%)	

The means from analyses of a group of 5-month-old hypophysectomized animals are given in the table below, while the individual rates are found in the table in the Appendix.

Table 14.
Completely hypophysectomized animals.

	Blood phosphorus mg. %		Blood calcium mg. %
M ± ε _m	5,1 ± 0,15/c.8*)/ 4,5 ± 0,15/c.20*)/		10,5 ± 0,21
	Plasma-protein %	Amount of urine ml./ 24 hrs.	Urinary phosphorus mg./24 hrs.
M ± ε _m	6.7 ± 0,14	19 ± 2,8/c.4*)/	5,4 ± 0,60/c.4*)

*) The figures within brackets indicate the number of days after the operation when the specimen was taken.

For the present, only the data pertaining to the blood phosphorus and blood calcium will be discussed. The blood phosphorus rates have been entered in two columns, showing the conditions 7—9 days and 17—25 days after the operation. Both rates are definitely below normal (5.8 ± 0.09 mg.%), but it is obvious that the depression of the phosphorus level extends by degrees over a considerable period. The blood calcium rates for the hypophysectomized animals show no definite divergency from normal.

Discussion.

The investigations described in this chapter have shown that, after administration of calf pituitary, the parathyroids of rats exhibit a slight but statistically valid enlargement. The enlargement is comparatively slight, however, as compared with that shown by the adrenals of those animals. As to the animals treated with pituitary gland, closer morphological examination discloses changes in the parathyroids which tally with over-activity, while the hypophysectomized animals present pictures indicating under-activity. So far the results might agree with the presence of a parathyrotropic factor in the pituitary gland. On the other hand, I was unable to demonstrate a definite diminution of the parathyroids after hypophysectomy. Both testes and adrenals however, presented a pronounced reduction in weight. The observation that there is no decrease in the size of the parathyroids after hypophysectomy hardly tallies with the conception of a pituitary factor stimulating the parathyroids of the same character as those which stimulate the testes and adrenal glands. Further, there is the behaviour of the blood phosphorus. If the enlargement which affects parathyroids after pituitary administration were due to the presence of a parathyrotropic factor in the glandular material, this factor would stimulate the parathyroids to over-activity. Under identical conditions otherwise, parathyroidal

over-activity produces a drop in the blood phosphorus and a rise in the blood calcium. However, the pituitary-treated animals show definitely raised blood phosphorus levels. On the other hand, in the hypophysectomized animals, owing to the absence of the alleged parathyrotropic factor, the function of the parathyroids would be depressed; the blood phosphorus would then rise and the blood calcium drop. Actually in these animals the blood phosphorus level is lowered, while the blood calcium is normal. The results recorded here can hardly be reconciled with the conception that the anterior lobe of the pituitary gland contains a factor which stimulates the parathyroids, in analogy with e. g. the testes and adrenals. It seems instead that the pituitary gland acts on the phosphorus metabolism, so that over-activity leads to an increase in blood phosphorus. Such a mechanism has been demonstrated clinically by, *inter alios*, Hurxthal (1948) in acromegalia. Li and his associates (1949) also reported that the reduction in phosphorus following hypophysectomy can be counterbalanced by administration of growth hormone from the anterior pituitary. Thus the pituitary gland acts on the blood phosphorus in such a way that over-activity produces an increase in the blood phosphorus, and inactivity depresses the blood phosphorus. The possibility of the parathyroidal function being affected by this mechanism has been pointed out earlier by Carnes (1943) and Törnblom (1949).

The result of the investigations detailed above can thus be satisfactorily explained in the following manner.

The pituitary gland contains a factor which elevates the blood phosphorus level. After pituitary administration the blood phosphorus rises, and as a result the parathyroids are stimulated to over-activity to counteract the rise in the phosphorus. If the stimulation of the organ is sufficiently powerful and prolonged, it entails hypertrophy. After hypophysectomy the blood phosphorus drops, viz. owing to the absence of the blood phosphorus-raising action of the pituitary gland. Consequently the parathyroidal function declines. The fact that no definite atrophy of the organ could be demonstrated in

the present investigation may be due, *inter alia*, to the drop in the blood phosphorus being rather slight and only gradually approaching the lowest rates (see also p. 75). Owing to the general nutritional disturbance brought about by the operation, and the considerable loss of weight ensuing from this, which must be expected to effect separate organs to different degrees, it is difficult to draw definite inferences. These investigations cannot supply definite information as to whether it is the change in the blood phosphorus as such, which affects the function of the parathyroids, or whether a possible, yet in the present study not demonstrated, simultaneous change in the calcium level may be the cause of the functional alteration.

One reason why research in this field as described in the historical survey, gave such divergent results is probably that the majority of workers attacked the problem from a purely chemical or purely morphological point of view, and that comprehensive morphological investigations were not supplemented by chemical studies.

Chapter 7.

THE BLOOD CHEMISTRY AFTER HYPOPHYSECTOMY COMBINED WITH PARATHYROIDECTOMY

To secure further evidence as to how the factors present in the parathyroids and pituitary, which affect the blood phosphorus, are interrelated, hypophysectomy and parathyroidectomy were performed simultaneously in a group of animals, the blood phosphorus being subsequently estimated after various periods within 3 weeks. The data are presented in the following table.

Table 15.

Inorganic phosphorus in the plasma in hypophys-parathyroid-
ectomized animals after:

4-7 days	9-12 days	15-21 days
7.7 mg. %	—	—
6.0 »	6.5 mg. %	—
7.1 »	—	—
7.9 »	6.1 »	—
6.2 »	6.0 »	—
6.1 »	—	—
5.7 »	—	—
7.0 »	—	—
—	7.0 »	6.7 mg. %
—	7.9 »	6.7 »
8.0 »	7.8 »	—
9.1 »	8.2 »	7.3 «
7.8 »	—	—
6.0 »	6.3 »	6.4 »
9.1 »	7.8 »	—
8.3 »	7.4 »	6.9 »

4-7 days	9-12 days	15-21 days
—	8.1 »	—
—	—	6.1 »
—	5.6 »	—
—	—	5.5 »
—	8.0 »	—
—	7.5 »	7.1 »
—	7.9 »	7.4 »
—	6.0 »	—
Totals 14	16	9
$M = 7.3 \pm 0.31$	$M = 7.1 \pm 0.23$	$M = 6.7 \pm 0.20$

For comparison, the corresponding rates for animals only parathyroidectomized were estimated, the following values being recorded.

Table 16.

20 parathyroidectomized animals

Blood phosphorus $\text{mg.}\%$	No. of days after the operation		
	2-3 days	10 days	20 days
M	7.4	8.1	8.3
ϵ_m	0.24	0.27	0.28

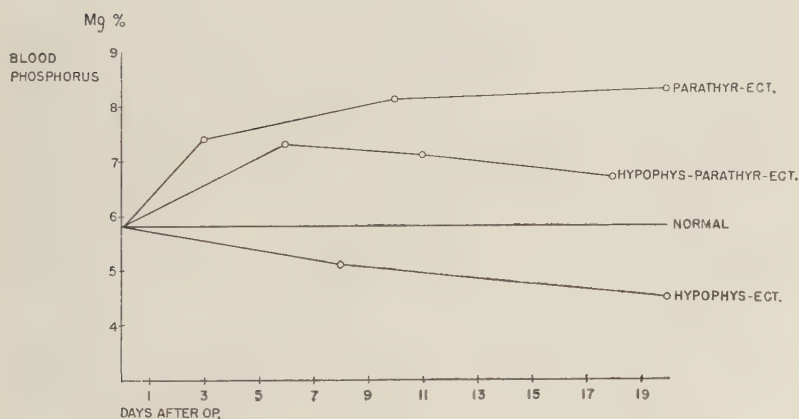


FIG. 9. Behaviour of the blood phosphorus after hypophysectomy combined with parathyroidectomy as compared with the findings after parathyroidectomy alone and after hypophysectomy alone.

The findings are further illustrated by Fig. 9. After combined hypophysectomy and parathyroidectomy the blood phosphorus level rises but not as high as after parathyroidectomy alone. After a steep initial elevation, rates show a tendency to drop. This tendency is noted after about 5—9 days, and after 20 days the phosphorus level is distinctly lower than it was 5 days after the operation. But even after 20 days the absolute figure is above normal.

Somewhat similar experiments with combined hypophysectomy and parathyroidectomy were described by Carnes and his associates (1943), who used rats, and by Törnblom (1949), who used rabbits. These workers did not keep their animals on a normal diet, however. Törnblom removed simultaneously the parathyroids, thyroid and pituitary gland from his rabbits, which had been given a diet abundant in calcium, and observed them for 6 days. He found no definite change in the blood phosphorus in these rabbits as compared with normal animals. On the other hand, in their rats, which were on a deficient supply of calcium, Carnes and his associates found a considerable rise in the blood phosphorus after combined hypophysectomy and parathyroidectomy. The rate of increase did not differ from that observed after parathyroidectomy alone.

In my investigation evidence was secured on the conditions produced by hypophys-parathyroidectomy by means of experiments arranged in a slightly different manner. The blood phosphorus was checked first, then the animals were hypophysectomized, and after a number of days the blood phosphorus was estimated again. Subsequently the parathyroids were removed, and after a further number of days a fresh check of the blood phosphorus was made. The animals were later given 20 units a day of growth hormone from the anterior pituitary lobe (phyol) on the subcutaneous route and the blood phosphorus was estimated. For the behaviour of the blood phosphorus, see Fig. 10. Thus hypophysectomy entails a drop in the blood phosphorus, which rises to slightly above normal after parathyroidectomy. Following administration of growth hormone, the phosphorus level is still more elevated.

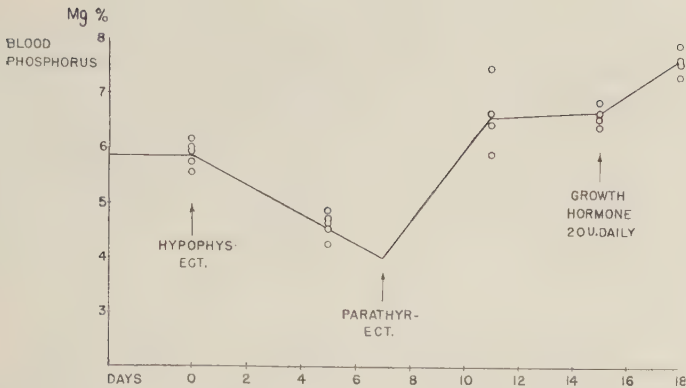


FIG. 10. Blood phosphorus variations after (first) hypophysectomy, (second) parathyroidectomy, and (third) administration of growth hormone.

The phosphorus-increasing effect of the growth hormone on hypophysectomized animals has been demonstrated earlier (Li and his colleagues). The effect of the growth hormone on the blood phosphorus of normal animals has been studied and described in the foregoing. Administration of this hormone to normal animals results in a definite increase in phosphorus. From the experiments described above it emerges that the growth hormone also has a phosphorus-increasing effect on animals which have been hypophysectomized. This observation suggests that, in that case, the growth hormone wholly or partly replaces the pituitary gland in respect of its phosphorus raising action. The above-described investigations concerning the effect of the pituitary and parathyroids on the blood phosphorus also indicate that they exercise their effect on the blood phosphorus independently of each other. Thus the effect of the pituitary on the blood phosphorus does not take place via the parathyroids. The results might possibly be taken to suggest a somewhat different mode of action of the pituitary gland and parathyroids as regards the phosphorus metabolism.

An interesting observation made on urinalysis of the hypophysectomized animals is, that there is a decrease in the amount of urine in these animals as compared

with those only hypophysectomized ($t = 3.28^{**}$ d. f. = 15). This findings supports the assumption that the parathyroids have a diuretic effect (*inter alios*, Shelling, 1938). The amount of phosphorus excreted per 24 hours by the hypophys-parathyroidectomized animals does not significantly differ from that excreted either by normal animals or by those only hypophysectomized (see the table below and Table X in the Appendix).

Table 17.
Hypophys-parathyroidectomized rats.

Per 24 hours	
Amount of urine ml.	Phosphorus in the urine mg.
4.0 (6)*	8.0
0.5 (6)	1.9
5.5 (5)	7.3
10.0 (5)	12.5
3.5 (6)	8.5
5.5 (4)	7.3
19.0 (4)	23.5
13.0 (4)	16.2
M = 7.5 ± 2.13	M = 10.7 ± 3.05

*) The figures within brackets indicate the number of days after the operation when the specimen was taken.

In this connection it is of interest to note that according to clinical observations polyuria is frequently an early symptom of osteitis fibrosa generalisata. This emerges, *inter alia*, from Hellström's (1950 Acta Chir. Scand., in the press) large and carefully examined case series of primary hyperparathyroidism. The renal injury which is often associated with hyperparathyroidism, viz. owing to the precipitation of calcium in the parenchyma, has been regarded as the cause of this polyuria, but the possibility of a direct effect of the parathyroid hormone has also been discussed. The supposition that the polyuria, at least in the cases of primary hyperthyroidism where renal injury cannot be definitely established, may be due to direct hormonal action, has obtained a certain support from the observations described above.

Chapter 8.

DOES THE PHOSPHORUS-RAISING ACTION OF THE PITUITARY GLAND DERIVE FROM THE ANTERIOR OR POSTERIOR LOBE?

The observations described in earlier chapters thus show that the pituitary gland exerts a phosphorus-increasing effect. It is then naturally of interest to ascertain which factor or factors are of importance in this respect. In general it has been assumed that the phosphorus-increasing effect of the pituitary gland is confined to the anterior lobe, which appears probable for several reasons. However, it has also been demonstrated that the posterior lobe hormone affects the phosphorus metabolism. Thus, Fraser (1949), *inter alios*, showed that the posterior lobe hormone produces a rise in the blood phosphorus. Therefore the possibility that, after hypophysectomy, a depression of the blood phosphorus level may be due to the loss of a phosphorus-increasing factor in the posterior lobe cannot simply be dismissed. Hence, in my opinion there are reasons for endeavouring to elucidate whether the posterior lobe plays a part in this respect. Two groups of animals were used for careful examination of the blood- and urinary chemistry. One group was completely hypophysectomized. In the other group the posterior lobe was removed and the anterior lobe left. Owing to the anatomical conditions it was inevitable that the anterior lobe should be injured to some extent, and small pieces of it were lost. However, the remaining piece was quite sufficient to maintain the function. This is made evident by the weight of the adrenals and testes,

which were not affected by the operation. The result will be found in the tables of means on p. 70 and below. For details, see the tables in the Appendix.

Table 18.

Hypophysectomized animals with the anterior lobe left.

Blood phosphorus mg. %	Blood calcium mg. %	Plasma-protein %
$5.9 \pm 0.30/8^*)$; $5.8 \pm 0.25/c.20^*)$	10.5 ± 0.12	7.0 ± 0.17
Amount of urine ml./24 hrs.	Urine phosphorus mg./24 hrs.	
$36 \pm 5.2/c.4^*)$	$8.8 \pm 1.37/c.4^*)$	

As will be seen, the hypophysectomized animals have distinctly reduced blood phosphorus rates, while the animals from which only the posterior lobe was removed have a normal blood phosphorus level. The blood calcium exhibits no definite changes in any of the groups, nor was any definite change in the plasma protein figures observed. The urine analyses afford some interesting observations. In the completely hypophysectomized animals the amount of urine excreted per 24 hours is considerably greater than normally (difference statistically valid), while the amount of phosphorus excreted keeps within normal limits. In the animals from which only the posterior lobe was removed the amount of urine excreted per 24 hours is very large and also larger ($t = 2.92^*$ d. f. = 16) than in the completely hypophysectomized animals. In this group the amount of phosphorus excreted per 24 hours does not significantly differ from the values for normal animals. Judging by the results of these investigations, it would appear that the depression of the blood phosphorus following on hypophysectomy is due to factors present in the anterior lobe, and, as the animals which have no posterior lobe do not show any change in their blood phos-

*) The figures within brackets indicate the number of days after the operation when the specimen was taken.

phorus, it appears hardly probable that normally the posterior lobe has any effect on the phosphorus in the blood. The great increase in the excretion of urine from the animals which have no posterior lobe tallies with the supposition that an intact anterior lobe is pre-conditional to the development of excessive diuresis on posterior lobe injury (Selye, 1948). However, the completely hypophysectomized animals also exhibit distinctly increased diuresis. No answer is afforded by these experiments to the question whether this is due only to the absence of the posterior lobe or is caused by injury to the hypothalamus during operation.

Chapter 9.

THE EFFECTS OF HORMONE ADMINISTRATION TO HYPOPHYSECTOMIZED ANIMALS

One effect of the growth hormone of the anterior pituitary lobe is to elevate the blood phosphorus. As has already been mentioned, the fact that the depression of the blood phosphorus after hypophysectomy is wholly or partly dependent on the absence of this factor in the anterior lobe has been demonstrated by Li *et al.*, who were able to bring about normalization of the blood phosphorus in hypophysectomized young rats by administering growth hormone. I have been able to verify these results with 150-day-old rats which had been hypophysectomized and for 10 days subsequently had been given 22 units a day of pure chystalline growth hormone (Wilhelmi, 1948). There was no depression of the blood phosphorus level, which was rather somewhat higher than normal.

No. of animals	$M \pm \epsilon_m$ mg. %
8	7.0 ± 0.17
(Normal rate 5.8 ± 0.09 mg. %)	

The parathyroids of the hypophysectomized rats which were given growth hormone were also studied morphologically. The picture does not tally with that of those only hypophysectomized. The ratio, cytoplasm to nucleus, was estimated and found to be lower than normal, but the nuclear surface was normal or possibly somewhat increased, and further, a number of mitoses were observed. These findings indicate a

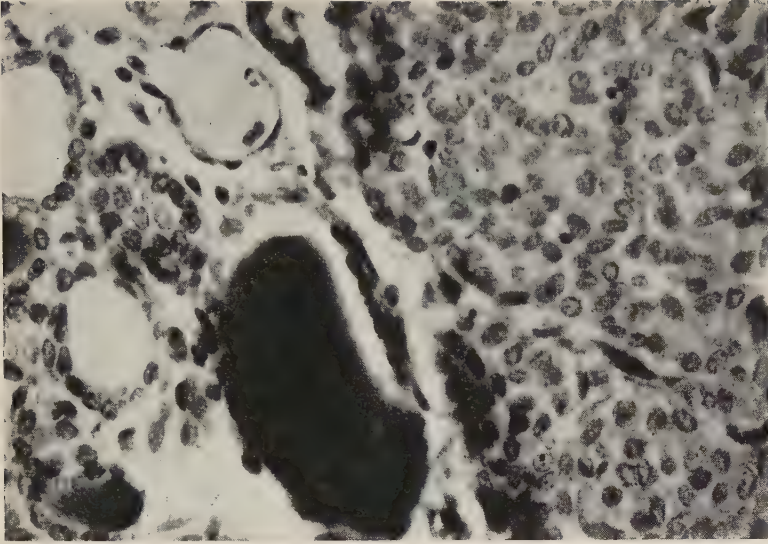


FIG. 11. Parathyroids, 5-month-old hypophysectomized rat which had been given 20 units of growth hormone daily for 10 days. (Note the low epithelium within the adjacent thyroid follicles which is a result of the hypophysectomy). $\times 500$.

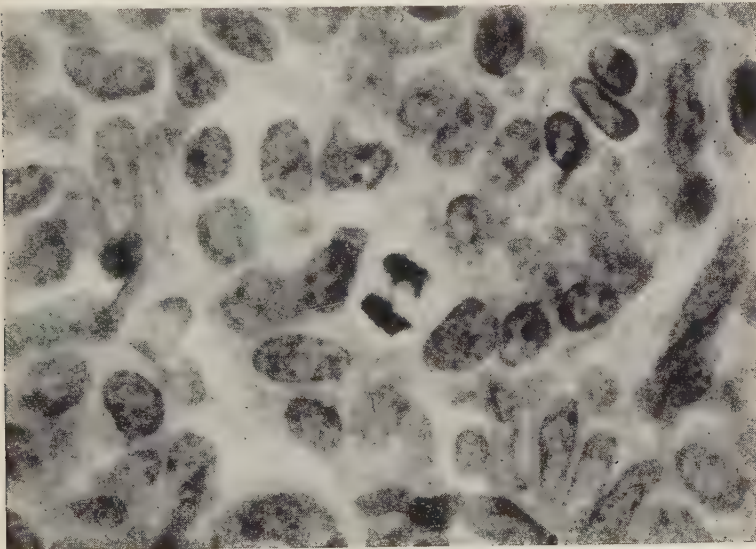


FIG. 12. Parathyroids, 5-month-old hypophysectomized rat which had been given 20 units of growth hormone daily for 10 days. (In the centre a mitosis). $\times 1350$.

normal, or perhaps rather an increased, function of the parathyroids of these animals (see table below).

Table 19.

Behaviour of nuclei and cytoplasm in the parathyroids of 5-month-old hypophysectomized rats after administration of growth hormone, ACTH, Eschatin and phosphorus for 10 days.

	No. of animals	Cytoplasm Nucleus $\pm \epsilon_m$	Nuclear surface*)	Cytoplasm surface*) calculated	Mitoses
Normal	7	M = 3.17 ± 0.058	M = 40.1	M = 126.9	0—2
Growth hormone	5	2.65 ± 0.16 1.97 ± 0.07 2.04 ± 0.13 2.15 ± 0.17 2.47 ± 0.14 M = 2.26 ± 0.069 t = 10.1*** d. f. = 142	50.1 50.8 48.5 52.3 44.9 M = 49.3	132.8 100.1 98.9 112.4 110.9 M = 111.0	8 2 1 12 3
ACTH	3	2.20 ± 0.15 2.08 ± 0.09 2.14 ± 0.16 M = 2.14 ± 0.077 t = 10.0*** d. f. = 118	37.6 35.3 36.7 M = 36.5	82.7 73.4 78.5 M = 78.2	0 0 0
Eschatin	1	2.26 ± 0.07 t = 5.8*** d. f. = 94	32.4	73.2	0
Phosphorus	4	2.96 ± 0.16 2.12 ± 0.13 2.20 ± 0.15 1.98 ± 0.14 M = 2.43 ± 0.090 t = 8.3*** d. f. = 130	40.9 38.6 42.6 45.9 M = 42.0	120.8 81.8 93.7 90.9 M = 96.8	6 2 4 11

*) Comparison figures, see *Materials and Methods*, p. 42.

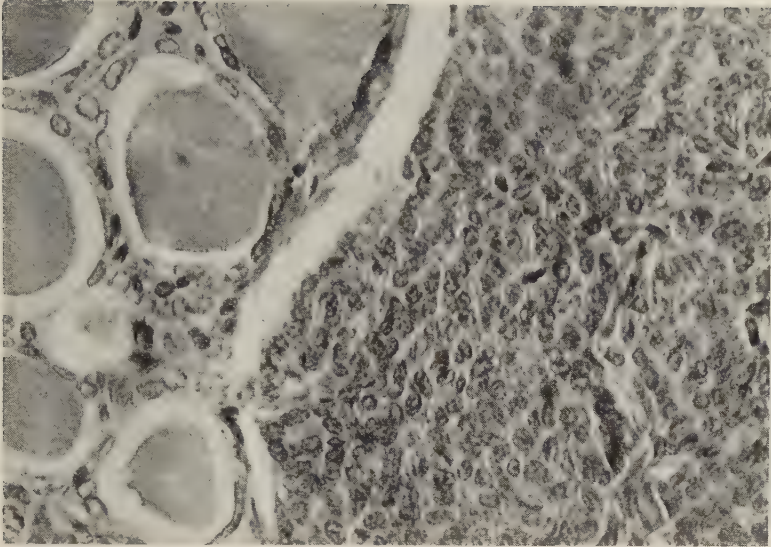


FIG. 13. Parathyroids, 5-month-old hypophysectomized rat which had been given 2 mg. ACTH daily for 10 days. $\times 500$.

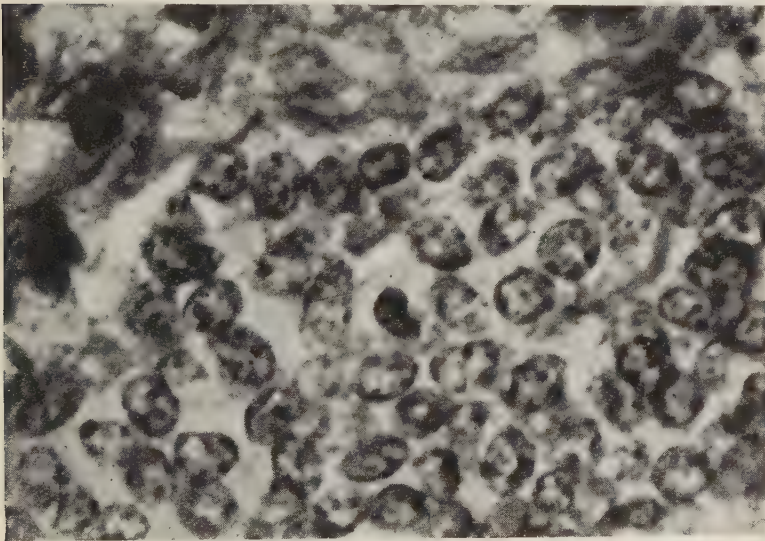


FIG. 14. The same section as in Fig. 13. $\times 1350$.

Apart from those with growth hormone, substitution experiments were carried out with ACTH, which was administered to the hypophysectomized animals in the dosage of 2 mg. daily. The preparation was supplied by the Organon Co. and is stated to contain traces of other substances. The parathyroids of these animals exhibited changes which tallied on the whole with those of the hypophysectomized animals (see table on p. 66). The blood phosphorus in 5 such animals presented figures which were at least as low as those for the untreated hypophysectomized animals ($4.5 \text{ mg. \%} \pm 0.2$). Some hypophysectomized rats were treated with the adrenal cortex extract Eschatin (0.5 ml. daily for 10 days), but their parathyroids also tallied with those of the animals that had been hypophysectomized only (see table on p. 66), and here, too, the blood phosphorus level was low. Thus the changes which develop in the parathyroids of hypophysectomized animals after the administration of growth hormone, could not be found in the animals which were given ACTH or adrenal cortex extract, nor could a corresponding rise in the blood phosphorus be demonstrated in these animals.

After administration of growth hormone from the anterior pituitary lobe an increase in the blood phosphorus is noted, and the parathyroids are also affected. The changes which arise in the parathyroids of hypophysectomized animals fail to develop if the animals are treated with growth hormone. It would then be of interest to investigate to what extent an increase in the blood phosphorus as a result only of a diet abundant in phosphorus or of parenteral administration of phosphate could affect the parathyroids of hypophysectomized animals. Some animals were therefore hypophysectomized and then put on a diet abundant in phosphorus for 10 days; they were also given 5 ml. a day of 2/15 normal phosphate buffer. After 10 days' treatment, these animals had slightly raised blood phosphorus rates. Their parathyroids presented changes signifying over-activity (see table on p. 52). The morphological picture exhibited by the parathyroids of these animals, thus tallies with those of the hypophysectomized animals

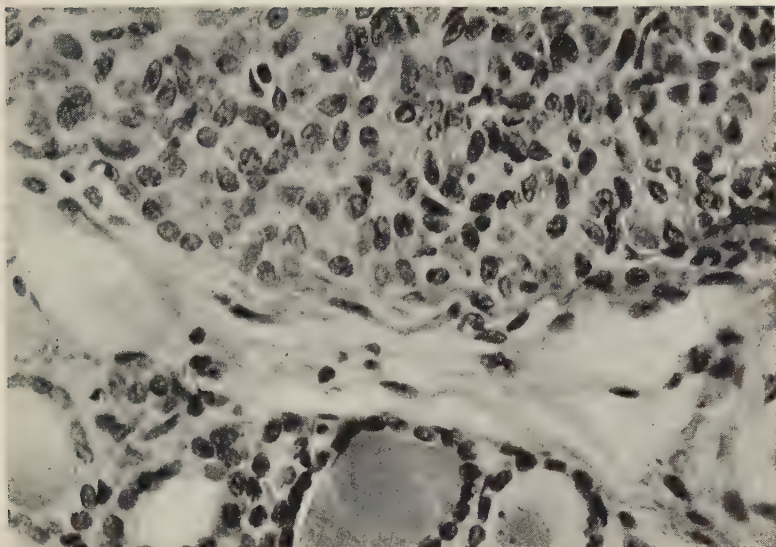


FIG. 15. Parathyroids, 5-month-old rat which had been given ample phosphorus for 10 days. $\times 500$.

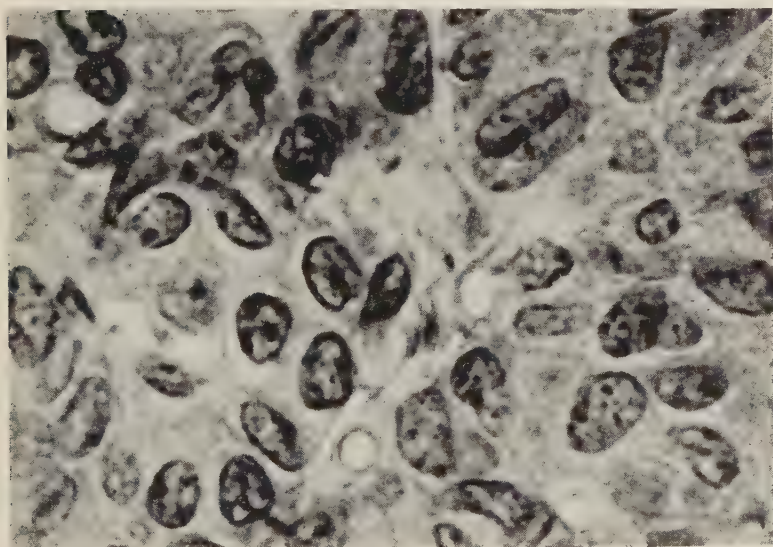


FIG. 16. The same section as in Fig. 15. $\times 1350$.

which had been given growth hormone. Hence, this change can be effected simply by supplying phosphorus to hypophysectomized animals. The experiments also show that parathyroidal over-activity can be induced in hypophysectomized rats by means of supplying them with abundant phosphorus, and thus that the parathyroids of these animals are not functionally insufficient.

Chapter 10.

INVESTIGATIONS CONCERNING THE RELATION OF THE PARATHYROIDS TO SOME OTHER ENDOCRINOUS ORGANS

The literature contains statements to the effect that the phosphorus-increasing action of the pituitary gland, *inter alia*, is restricted to the corticotropic hormone and is transmitted via the adrenals (Törnblom, 1949). If the adrenal cortex had a phosphorus-increasing effect, removal of the adrenals ought to depress the blood phosphorus. This aspect of the problem was investigated with different groups of 5-month-old rats whose adrenals had been removed. After 6 days one group of animals, which had had no treatment of any kind after the operation, presented the following blood phosphorus rate.

No of animals	$M \pm \epsilon_m$ m. %
12	7.8 ± 0.16
(Normal rate 5.8 ± 0.09 mg. %)	

In another group 3 ml. normal saline was injected daily on the subcutaneous route. A third group received daily 1/10 mg. adrenalin subcutaneously in 3 doses. A fourth group was treated with daily 0.5 ml. of doca subcutaneously. In all the cases the animals presented definitely raised phosphorus rates after 6 days. The blood calcium rate in the untreated rats which had had their adrenals removed was 9.9 ± 0.26 mg.% (normal rate 11.3 ± 0.12 mg.%). After combined parathyroidectomy and adrenalectomy the blood phosphorus rises to

levels which are higher than those found after either operation alone.

No. of animals	$M \pm \epsilon_m$ mg. %
9	8.7 ± 0.5

On morphological examination of the parathyroids of the animals whose adrenals had been removed, an increased number of mitoses and a depressed ratio, cytoplasm to nucleus, as well as an increased nuclear surface were found. These findings suggested parathyroidal over-activity (see the table below).

Table 20.

Behaviour of nuclei and cytoplasm in the parathyroids of 5-month-old rats 6 days after the adrenals have been removed.

	No. of animals	Cytoplasm Nucleus $\pm \epsilon_m$	Nuclear surface*)	Cytoplasmic surface*) (calculated)	Mitoses
Normal	7	$M = 3.17 \pm 0.058$	$M = 40.1$	$M = 126.4$	0—2
Adrenal-ectomized	4	2.28 ± 0.07	44.0	100.3	15
		1.96 ± 0.10	50.1	98.2	4
		2.10 ± 0.06	50.3	105.6	8
		2.42 ± 0.06	48.2	116.6	7
		$M = 2.19 \pm 0.044$	$M = 48.2$	$M = 105.2$	
		$t = 11.7^{***}$			
		d. f. = 130			

*) Comparison figures, see *Materials and Methods*, p. 42.

Blumenfeld and Clausen (1940), who demonstrated that the blood calcium drops after removal of the adrenals, have examined the volume of the parathyroids after that operation, and they state that they found a slight — but not statistically valid — decrease in the volume of the organ. They interpret their findings as indicating that the parathyroidal function is reduced rather than otherwise after adrenalectomy. My investigations show, however, that the changes in the blood che-

mistry following adrenalectomy stimulate the parathyroids to over-activity. Neither these data, nor the results of experiments with ACTH or Eschatin discussed in the preceding chapter, afford any support for the supposition that the phosphorus-increasing effect of the pituitary gland is transmitted via the adrenals.

A number of workers are of the opinion that there is a certain connection between the parathyroidal function and the pancreas. *Houssay* et al. (1933) after pancreatectomy found regressive changes in the parathyroids of dogs, while *Mentha* (1941) noted hyperplasia of the parathyroids in dogs with experimental diabetes. The volume of the parathyroids in a small series of experimental diabetes in rats, after removal of the pancreas, has been studied (material from unpublished paper by *Engfeldt*, *Hultquist* and *Ohlsson*).

No. of animals	Volume in cu. mm.	Volume/Gm. body weight
	$M \pm \epsilon_m$	$M \pm \epsilon_m$
8	0.1882 ± 0.0166	0.00140 ± 0.00025

The age of the rats was about 2 months at the time of the operation and fully 3 month at death. Thus in these animals a definite enlargement of the parathyroids is found (for comparison, see the normal material, Chap. 4). This observation may be viewed against the background of the well-known fact that in cases of untreated diabetes mellitus there is often a rise in the blood phosphorus.

Chapter 11.

GENERAL SURVEY OF RESULTS

By way of introduction, an account was given of the normal macroscopic and microscopic aspects of the parathyroids of rats in different age-groups, starting from the new-born. The observations made concerning the size of the organ in different age-groups tally well with the statements found in the literature.

My results do not afford any support for the view that the size of the cells in the parathyroids decreases after the onset of puberty. It appears rather that, if there is any change at all in the size of the cells, it is in the direction of larger cells in the sexually mature rats than in younger ones.

It has not been possible to identify the different cell types in the parathyroids which have been described. It would appear to the present writer that there is only one cell type, viz. the so-called chief cells.

As regards the blood chemistry of normal rats, it will only be pointed out that before puberty the blood phosphorus is somewhat higher than in the sexually mature animals.

Attempts have been made with the aid of cytological methods to secure early and accurate evidence of changes in the functional state of the parathyroids. The ratio, cytoplasm to nucleus, and the nuclear surface have been calculated in different functional states of the parathyroids. On making a comparison with normal animals of corresponding ages, we find characteristic changes associated with both over-activity and under-activity of the organ. With increased function, a

reduction of the ratio, cytoplasm to nucleus, is observed, and the nuclear surface increases absolutely, while the cytoplasmic surface remains roughly stable. Depressed parathyroidal function entails also a lower ratio, but in this case the nuclear surface diminishes, from which it follows that the cytoplasmic surface decreases relatively still more than the nuclear surface. In comparative experiments with simultaneous investigation of the features alluded to here and determinations of the total size of the organ, I was able to show that the cytological changes which indicate over-activity can be observed long before it is possible to demonstrate a definite enlargement of the organ. Further, it requires a number of observations to establish enlargement of the parathyroids, while in suitable instances one single cytological investigation can provide information as to the functional state.

Information has been pursued in different ways concerning the possible existence of a parathyrotropic factor in the pituitary gland. In the first place, morphological studies were carried out (volume determinations of the organ and cytological examinations), and in the second, blood chemical details were studied. The results having been discussed fairly exhaustively in Chapter 5, it will suffice to state here that the findings cannot be satisfactorily explained on the grounds of a parathyrotropic factor in the anterior pituitary lobe. On the contrary, it may be considered established that no purely parathyrotropic factor in the same sense as, for instance, the corticotropic factor, can be demonstrated. However, there are possibilities of a functional connection between the parathyroids and the pituitary, viz. via the action of these glands on the blood chemistry. Thus in the anterior pituitary lobe there is a factor (or possibly several factors) which has the effect of directly or indirectly elevating the blood phosphorus. If the pituitary gland is over-active, the blood phosphorus rises, and as a result of the rise in blood phosphorus, the parathyroids are forced to intensify their function in order to counteract that rise.

The relation in respect of the action on the blood phos-

phorus of the pituitary gland and the parathyroids has been studied by means of combined hypophysectomy and parathyroidectomy. In my investigations, in which the animals were given a normal diet and observed for up to 3 weeks, a rise in blood phosphorus followed on hypophys-parathyroidectomy, which, however, was not as high as after parathyroidectomy alone. After an initial rise, the blood phosphorus also presented a falling tendency. I have interpreted these observations as showing that the blood-phosphorus-increasing effect following on parathyroidectomy is as powerful as otherwise, even if the pituitary gland has been removed at the same time. However, as a result of the simultaneous hypophysectomy, a blood-phosphorus-decreasing factor asserts itself, and, as has been shown, the blood-phosphorus-decreasing effect of hypophysectomy is slow to develop to the full extent. In that way the modified blood phosphorus curve after hypophys-parathyroidectomy may conceivably present itself as a summation of the two blood phosphorus curves for parathyroidectomy and hypophysectomy separately.

Hypophys-parathyroidectomy entails a reduced excretion of urine as compared with hypophysectomy alone. This observation is evidence in favour of a diuretic action of the parathyroids.

The blood phosphorus increase produced by administration of growth hormone to the hypophys-parathyroidectomized animals, supports for the assumption that the phosphorus-increasing factor in the anterior pituitary lobe acts independently of the parathyroids.

That the parathyroids in the hypophysectomized animals are not functionally insufficient is shown, *inter alia*, by experiments with administration of phosphorus to hypophysectomized animals, whose parathyroids then present pictures that tally with that of an over-active gland.

Substitution experiments with pure growth hormone on hypophysectomized animals show that this hormone will check the drop in blood phosphorus which ensues from hypophys-

ectomy. The parathyroids of such animals exhibit a normal picture or signs of over-activity.

With regard to the morphological changes in the parathyroids of hypophysectomized animals which had received ACTH and adrenal cortex extract, it can be demonstrated that the picture is not that shown by normal animals, nor does it tally with that of an over-active gland, but is of the same type as that met with in those only hypophysectomized. Nor, in the case of these animals, do the blood phosphorus level show any definite divergencies from that of the untreated hypophysectomized animals.

Adrenalectomy entails elevation of the blood phosphorus level, and this rise is hardly due to a reduced parathyroidal function. Owing to the absence of the adrenals, the blood chemistry is altered, and consequently the parathyroids are forced to increase their function. This explanation is supported by the fact that the morphological changes in the parathyroids following adrenalectomy were identical in type with those of an over-active gland.

The observations made concerning the morphology of the parathyroids of hypophysectomized animals which had been given ACTH or Eschatin, and of animals whose adrenals had been removed, and, further, the behaviour of the blood phosphorus in these animals, do not afford any support for the view that the phosphorus-increasing effect of the pituitary gland is transmitted via the adrenals.

Rats with diabetes following on extirpation of the pancreas exhibit a definite enlargement of the parathyroids, and this enlargement may probably be connected with the disturbances in the phosphorus metabolism associated with diabetes.

Summary.

The present study, based on experiments with animals, comprises combined morphological, blood-chemical, and urine-chemical investigations carried out, with the object of gaining information as to the factors governing the parathyroidal function.

1. The size of the parathyroids was estimated in albino rats of different ages.

2. The ratio, cytoplasm to nucleus, and the nuclear surface were determined for normal animals of different age-groups.

3. The blood phosphorus, blood calcium and plasma protein rates were estimated in normal animals, and the amount of urine per 24 hours and the amount of phosphorus excreted were recorded.

4. The ratio, cytoplasm to nucleus, and, further, the nuclear surface were estimated at different functional states of the parathyroids, and were found to show characteristic changes associated with over- and under-activity of the organ.

5. The cytological changes appear a considerable time before a change in the size of the organ can be demonstrated.

6. The investigations mentioned in the paragraphs 1—3 were carried out in pituitary-treated and hypophysectomized animals, and as the result of these experiments the following conclusions were drawn:

a) No parathyrotropic factor in the ordinary sense is found in the anterior pituitary lobe.

b) The pituitary gland does affect the parathyroidal function, however, but this action is indirect, viz. via the effect of the pituitary on the blood chemistry.

c) The pituitary gland has a phosphorus-increasing action which is confined to the anterior lobe, since the drop in blood phosphorus ensuing from hypophysectomy is absent if the posterior lobe only is removed.

7. Administration of growth hormone to hypophysectomized rats entails a rise in the blood phosphorus to normal

or beyond and morphological signs of normal or increased parathyroidal function.

8. ACTH and adrenal cortex extract (Eschatin) which were given to hypophysectomized animals failed to prevent the morphological changes which develop in the parathyroids after hypophysectomy and did not restore the low blood phosphorus.

9. Hypophys-parathyroidectomy leads to an increase in blood phosphorus, which initially is fairly considerable but not so high as after parathyroidectomy alone. The blood phosphorus level of hypophys-parathyroidectomized rats shows a gradual tendency to drop, but does not attain a normal rate.

10. The excretion of urine by the hypophys-parathyroidectomized rats is not definitely increased as compared with that of normal animals, but is certainly less than after hypophysectomy alone.

11. Adrenalectomy entails elevation of the blood phosphorus level.

12. The parathyroids of adrenalectomized animals present pictures suggesting over-activity.

13. The parathyroids of rats with experimental pancreatic diabetes are distinctly enlarged.

REFERENCES.

- F. Albright, W. Bauer, M. Ropes, J. C. Aub: J. Clin. Investigation 7:139, 1929.
- F. Albright, R. Ellsworth: J. Clin. Investigation 7:183, 1929.
- F. Albright, J. Aub, W. Bauer: J. A. M. A. 102:1276, 1934.
- F. Albright, T. G. Drake, H. W. Sulkowitch: Bull. Johns Hopkins Hosp. 60:377, 1937.
- F. Albright, H. W. Sulkowitch, E. Bloomberg: J. Clin. Investigation 18:165, 1939.
- F. Albright: J. A. M. A. 117:527, 1941.
- F. Albright: J. Clin. Investigation 20:445, 1941.
- F. Albright: Glandular physiology and therapeutics. Chicago P. 443, 1942.
- F. Albright, C. H. Brunett, P. H. Smith, W. Parson: Endocrinology 30:922, 1942.
- F. Albright: J. Clin. Endocrinology 8:637, 1948.
- F. Albright, E. Reifenshtein: Baltimore 1948.
- N. Alwall: Acta Med. Scand. 116:337, 1944.
- A. B. Anderson, E. F. Oastler: J. Physiol. 92:124, 1938.
- K. J. Anselmino, Fr. Hoffman, L. Herold: Klin. Wochnschr. 12:1944, 1933.
- K. J. Anselmino, Fr. Hoffman: Klin. Wochnschr. 13:44, 1934.
- K. J. Anselmino, Fr. Hoffman, L. Herold: Klin. Wochnschr. 13:45, 1934.
- K. J. Anselmino, L. Herold, Fr. Hoffman: Ztschr. f. d. ges. exper. Med. 97:51, 1935/36.
- B. Aschner: Arch. f. d. ges. Physiol. /Pflüger's/ 146:86, 1912.
- M. Askanazy: Arb. a. d. Geb. d. path. Anat. Inst. zu Tübing., Leipzig 4:398, 1904.
- E. Ask-Upmark: Acta Med. Scand. 74:284, 1930/31.
- J. C. Aub, W. Bauer, C. Heath, M. Ropes: J. Clin. Investigation 7:97, 1929.
- J. C. Aub, F. Albright, W. Bauer, E. Rossmeisl: J. Clin. Investigation 11:211, 1932.
- B. L. Baker: Anat. Rec. 83:47, 1942.
- B. L. Baker: Anat. Rec. 93:125, 1945.

- B. L. Baker, D. J. Ingle, C. H. Li, H. M. Evans: *Anat. Rec.* 102:313, 1948.
- D. P. Barr, H. A. Bulger, H. H. Dixon: *J. A. M. A.* 92:951, 1929.
- P. D. Bartlett, O. H. Gaebler: *Endocrinology* 43:329, 1938.
- P. Bastenie, S. Zylberszac: *Comt. rend. Soc. de biol.* 127:822, 1938.
- P. Bastenie, S. Zylberszac: *Comt. rend. Soc. de biol.* a/ 132:93, b/ 132:95, 1939.
- W. Bauer, J. C. Aub: *J. Clin. Investigation* 20:295, 1941.
- E. J. Baumann, D. B. Sprinson: *Am. J. Physiol.* 125:741, 1939.
- J. Benoit, G. Fabiani, R. Grangaud, J. Clavert: *Comt. rend. Soc. de biol.* 135:1606, 1941.
- J. Benoit, J. Clavert, R. Cabannes: *Comt. rend. Soc. de biol.* 138:1071, 1944.
- H. Bergstrand: *Acta Med. Scand.* 52:791, 1919/20.
- H. Bergstrand: *Acta Med. Scand.* 54:539, 1921.
- H. Eergstrand: *Acta Med. Scand.* 76:128, 1931.
- H. Bergstrand: *Acta path. et mikrobiol. Scand. suppl.* 38-40:80, 1938/39.
- H. Bergstrand: *Acta Chir. Scand.* 85:25, 1941.
- R. Berlin: *Acta Med. Scand.* 135:18, 1949.
- A. Biedl: *Innere Sekretion*, Berlin 1916.
- A. Biering: *Acta Paediat.* 31:235, 1943.
- A. Biering: *Diss. Universitetsforlaget Aarhus* 1950.
- C. M. Blumenfeld, H. M. Rice: *Anat. Rec.* 70:227, 1937/38.
- C. M. Blumenfeld, F. W. Clausen: *Am. J. Physiol.* 128:577, 1940.
- H. T. Blumenthal, L. Loeb: *Endocrinology* 30:502, 1942.
- A. Eodansky, J. E. Blair, H. L. Jaffe: *Proc. Soc. Exper. Biol. a. Med.* 27:708, 1929/30.
- M. Bodansky, V. B. Duff: *J. Nutrition* 21:179, 1941.
- M. Bodansky, V. B. Duff: *J. Nutrition* 21:235, 1941.
- M. Bodansky, V. B. Duff: *J. Nutrition* 22:25, 1941.
- A. Bolliger, F. W. Hartman: *J. Biol. Chem.* 64:91, 1925.
- C. Bomskov: *Metodik der Hormonforschung*, Leipzig 1937.
- G. Bonnier, O. Tedin: *Biologisk Variationsanalys*, Stockholm 1940.
- F. Brink Jr., D. W. Bronk: *Proc. Soc. Exper. Biol. a. Med.* 37:94, 1937.
- S. E. Brolin: *Acta Endocrin.* 1:304, 1948.
- M. Brown, C. G. Imrie: *J. Physiol.* 75:366, 1932.
- L. Brull, G. Carbonesco: *Compt. rend. Soc. de biol.* 131:800, 1939.
- R. B. Burrows: *Am. J. Anat.* 62:237, 1937.
- I. L. Campbell, C. W. Turner: *Research Bulletin Columbia, Missouri* 1942.
- L. Cannavò: *Biochem. Ztschr.* 245:234, 1932.
- L. Cannavò, R. Beninato: *Endokrinologie* 15:389, 1935.

- A. Cantarow, H. C. Stewart, E. L. Housel: *Endocrinology* 22:13, 1938.
A. Cantarow, V. G. Haury: *Am. J. Physiol.* 126:66, 1939.
W. H. Carnes, A. M. Pappenheimer, H. C. Stoerk: *Proc. Soc. Exper. Biol. a. Med.* 51:314, 1942.
W. H. Carnes, J. Osebold, H. C. Stoerk: *Am. J. Physiol.* 139:188, 1943.
M. S. Carttar, T. C. McLean, M. R. Urist: *Am. J. Path.* 26:307, 1950.
T. Caspersson, Hj. Holmgren: *Anat. Anzeiger* 79:53, 1934/35.
T. Caspersson, H. Landström-Hydén, L. Aquilonius: *Chromosoma* 2:111, 1941.
T. Caspersson: *Cell growth and cell function*, New York 1950.
B. Castleman, T. B. Mallory: *Am. J. Path.* 11:1, 1935.
B. Castleman, T. B. Mallory: *Am. J. Path.* 13:553, 1937.
M. Cattaneo: *Riv. di pat. spes.* 20:361, 1938.
H. W. Chalkley: *J. Nat. Cancer. Inst.* 4:47, 1943/44.
E. P. Clark, J. B. Collip: *J. Biol. Chem.* 63:461, 1925.
W. E. Cohn, E. T. Cohn, J. C. Aub: *Ann. Rev. Biochem.* 11:415, 1942.
J. B. Collip: *J. Biol. Chem.* 63:395, 1925.
J. B. Collip, E. P. Clark, J. W. Scott: *J. Biol. Chem.* 63:439, 1925.
J. B. Collip, J. Mount: *J. Mt. Sinai Hosp.* 1:28, 1934.
J. B. Collip, L. J. Pugsley, H. Selye, D. L. Thomson: *Brit. J. Exper. Path.* 15:335, 1934.
J. B. Collip: *J. A. M. A.* 115:2073, 1940.
O. Cope: *Clinics* 1:1168, 1942/43.
J. Courjaret, J. Poujol: *Le Bulletin Médical* 62:333, 1948.
W. Cox Jr., M. Imboden: *J. Nutrition* 11:147, 1936.
H. Cushing, L. M. Davidoff: *Monographs of the Rockefeller institute for medical research* nr 22, 1927.
H. Cushing: *Arch. of Int. Med.* 51:487, 1933.
J. F. Danielli: *J. of Exp. Biol.* 22:110, 1946.
E. W. Dempsey, R. O. Greep, H. W. Deane: *Endocrinology* 44:88, 1949.
M. Desclin: *Bull. Acad. roy. de méd. de Belgique* 8:340, 1943.
T. F. Dixon: *Biochem. J.* 27:410, 1933.
L. R. Dragstedt: *Physiol. Rev.* 7:499, 1927.
T. G. Drake, F. Albright, B. Castleman: *J. Clin. Investigation* 16:203, 1937.
A. ver Eecke: *Arch. internat. de pharmacod.* 4:81, 1898.
R. Ellsworth, P. H. Fitcher: *Bull. Johns Hopkins Hosp.* 57:91, 1935.
B. Engfeldt: *Svenska Läkartidningen* 23:1271, 1949.
B. Engfeldt: *Nord. Med.* 43:921, 1950.
J. Erdheim: *Beitr. z. path. anat. u. zu allg. Path.* 33:158, 1903.
J. Erdheim: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* 16:632, 1906.
J. Erdheim: *Sitz. d. k. Acad. d. Wiss. Wien Math. Naturwissen*

- 3:311, b 66, 1907.
- J. Erdheim: Frankf. Ztschr. f. Path. 7:175, 1911.
- J. Erdheim: Wien 1914.
- H. M. Evans, M. E. Simpson, C. H. Li: Growth 12:15, 1948.
- A. Fagreus: Acta med. scand. suppl. 204, 1948.
- M. Fay, V. G. Behrmann, D. M. Buch: Am. J. Physiol. 136:716, 1942.
- C. H. Fiske, Y. Subbarow: J. Biol. Chem. 66:375, 1925.
- C. L. Foster: J. Endocrinol. 3:244, 1942/44.
- A. M. Fraser: J. Physiol. 108:345, 1949.
- S. Freeman, T. S. Chang: Am. J. Physiol. 160:341, 1950.
- J. S. Friedenwald, J. E. Crowell: Bull. Johns Hopkins Hosp. 84:568, 1949.
- H. B. Friedgood, R. McLean: Am. J. Physiol. 118:588, 1937.
- C. A. Gemzell, L. T. Samuels: Endocrinology 47:48, 1950.
- R. Gerschman: Compt. rend. Soc. de biol. 108:494, 1931.
- R. Gerschman, A. D. Marenzi: Compt. rend. Soc. de biol. 120:817, 1935.
- M. E. Gley: Compt. rend. Soc. de biol. 43:841, 1891.
- Kl. Gollwitzer-Meier: Ztschr. f. d. gez. exper. Med. 51:466, 1926.
- G. Gomori: Proc. Soc. Exper. Biol. a. Med. 72:449, 1949.
- A. L. Grafflin: Endocrinology 26:857, 1940.
- A. L. Grafflin: Endocrinology 30:571, 1942.
- F. S. Greenspar, C. H. Li, M. E. Simpson, H. M. Evans: Endocrinology 45:455, 1949.
- J. Greenwald: Am. J. Physiol. 29:103, 1911.
- I. Greenwald: J. Biol. Chem. 14:269, 1913.
- I. Greenwald: J. Biol. Chem. 61:33, 1924.
- I. Greenwald, J. Gross: J. Biol. Chem. 66:185, 1925.
- I. Greenwald, J. Gross: J. Biol. Chem. 66:217, 1925.
- I. Greenwald: J. Biol. Chem. 67:1, 1926.
- I. Greenwald: J. Biol. Chem. 68:325, 1926.
- W. E. Griesbach, T. H. Kennedy: Brit. J. Exp. Path. 30:555, 1949.
- J. Q. Griffith, E. J. Farris: Montreal 1942.
- A. W. Ham, R. E. Haist: Nature 144:835, 1939.
- A. W. Ham: Anat. Rec. 2:89, suppl. 76, 1940.
- A. W. Ham, N. Littner, T. G. H. Drake, E. C. Robertson, F. F. Tisdall: Am. J. Path. 16:277, 1940.
- B. Hamilton, Ch. Schwartz: J. Pharm. Exp. Therap. 46:285, 1932.
- R. R. Hannon, E. Shorr, W. S. McClellan, E. F. DuBois: J. Clin. Investigation 8:215, 1930.
- A. M. Hanson: Military surgeon 1:218, 1924.
- H. E. Harrison, H. C. Harrison: Am. J. Physiol. 134:781, 1941.
- H. E. Harrison, H. C. Harrison: J. Clin. Investigation 20:47, 1941.
- H. E. Harrison, H. C. Harrison: Am. J. Physiol. 134:781, 1941.

- A. J. Helfet: *Brit. J. Surg.* 27:651, 1939/40.
- J. Hellström, F. Wahlgren: *Acta Chir. Scand.* 91:472, 1944.
- J. Hellström: *Acta Chir. Scand.* 1950 in the press.
- O. E. Helve: *Biochem. Ztschr.* 306:341, 1940.
- J. B. Herrman, E. Kirstein, J. S. Krakauer: *J. Clin. Endocrinology* 9:1, 1949.
- S. Hertz, A. Kranes: *Endocrinology* 18:350, 1934.
- F. Hoff: *Verh. Deutsch. Ges. inn. Med.* 46:441, 1934.
- L. Hogben, E. Charles, D. Slome: *J. of Exp. Biol.* 8:345, 1931.
- L. Hogben, E. Charles: *J. of Exp. Biol.* 9:139, 1932.
- M. M. Hoskins: *Endocrinology* 8:777, 1924.
- M. M. Hoskins, S. B. Chandler: *Anat. Rec.* 30:95, 1924/25.
- B. C. Houghton, K. P. Klassen, G. M. Curtis: *Ohio Stat. Med. J.* 35:505, 1939.
- B. A. Houssay, P. Mazzocco: *Compt. rend. Soc. de biol.* 86:409, 1922.
- B. A. Houssay, R. Sammartino: *Compt. rend. Soc. de biol.* 114:729, 1933.
- B. A. Houssay, R. Sammartino: *Beitr. z. path. anat. u. z. allg. Path.* 93:405, 1934.
- B. A. Houssay: *New England J. Med.* 214:1128, 1936.
- H. Huber: Thèse, Université de Lausanne 1949.
- W. Hueper: *Arch. Path.* 3:14, 1927.
- L. M. Hurxthal: *Lahey Clin. Bull.* 5:194, 1948.
- L. M. Hurxthal, H. F. Hare, G. Horrax, J. L. Poppen: *J. Clin. Endocrinology* 9:126, 1949.
- H. Hydén: *Acta physiol. scand. suppl.* 17, 1943.
- B. Hölscher: *Pflüger's Arch. f. d. ges. Physiol.* 249:731, 1947/48.
- T. Ichijo: *Jap. J. M. Sc. Tr. IV, Pharmacol.* 8:85, 1934.
- C. G. Imrie, C. W. Jenkinson: *J. Physiol.* 79:218, 1933.
- T. H. Ingalls, G. Donaldson, F. Albright: *J. Clin. Investigation* 22:603, 1943.
- C. M. Jackson: *Am. J. Anat.* 19:305, 1916.
- H. L. Jaffe, A. Bodansky: *J. Exper. Med.* 52:669, 1930.
- H. L. Jaffe, A. Bodansky, J. E. Blair: *J. Exper. Med.* 55:139, 1932.
- H. L. Jaffe: *Arch. Path.* 16:63, 1933.
- I. Jahan, R. Pitts: *Am. J. Physiol.* 155:42, 1948.
- W. A. Jarrett, H. L. Peters, A. M. Pappenheimer: *Proc. Soc. Exper. Biol. a. Med.* 32:1211, 1934/35.
- L. M. Jones, G. Y. Shinowara: *J. Biol. Chem.* 142:935, 1942.
- T. Kemp, L. Marx: *Acta path. et microbiol. Scand.* 14:197, 1937.
- E. J. King: London 1946.
- L. W. Kinsell, G. D. Michaels, C. H. Li, W. E. Larsen: *J. Clin. Endocrinology* 8:1013, 1948.
- K. Kobayashi: *Jap. J. M. Sc. Tr. IV Pharmacol.* 5:56, 1931.

- S. Koster, A. Geesink: *Pflüger's Arch. f. d. ges. Physiol.* 222:293, 1929.
- S. Koster: *Pflüger's Arch. f. d. ges. Physiol.* 224:212, 1930.
- D. B. Kroon: *Endocrinology* 2:227, 1949.
- G. Kusunoki: *Fol. Endocrinologica Japonica* 3:34, 1927.
- S. Lagerstedt: *Diss. Lund* 1949, *Acta Anat. suppl.* IX.
- R. Levine, S. D. Loube, H. F. Weisberg: *Am. J. Physiol.* 159:107, 1949.
- M. V. L'Heureux, W. R. Tweedy, E. M. Zorn: *Proc. Soc. Exp. Biol. a. Med.* 71:729, 1949.
- C. H. Li, H. M. Evans: *Recent progress in hormon research vol. III.*
- C. H. Li, C. Kalman, H. M. Evans: *J. Biol. Chem.* 169:625, 1947.
- C. H. Li, D. J. Ingel, H. M. Evans, M. C. Prestruda, J. E. Negarnis: *Proc. Soc. Exp. Biol. a. Med.* 70:753, 1949.
- C. H. Li, I. Geschwind, H. M. Evans: *Endocrinology* 44:67, 1949.
- Ch. Livon, Peyron: *Compt. rend. Soc. de biol.* 71:49, 1911.
- J. Loeb: *Am. J. Physiol.* 3:383, 1900.
- M. A. Logan: *J. Biol. Chem.* 127:711, 1939.
- M. A. Logan, W. R. Christenson, J. W. Kirklin: *Am. J. Physiol.* 135:419, 1942.
- E. M. Luce: *J. Path. a. Bact.* 26:200, 1923.
- H. Lucke, E. Heckmann: *Arch. für exp. Path.* 87:189, 1938.
- W. G. McCallum, C. Voegtlin: *Bull. Johns Hopkins Hosp.* 19:91, 1908.
- W. G. McCallum, C. Voegtlin: *J. Exper. Med.* 11:118, 1909.
- F. A. McJunkin, W. R. Tweedy, E. W. McNamara: *Am. J. Path.* 13:325, 1937.
- F. C. McLean, A. B. Hastings: *J. Biol. Chem.* 108:285, 1935.
- F. C. McLean, W. Blom: *Science* 85:24, 1937.
- J. v. Magyary-Kossa: *Pflüger's Arch.* 245:547, 1942.
- J. Malcolm, W. E. Griesbach, F. Bielschowsky, W. H. Hall: *Brit. J. Exper. Path.* 30:17, 1949.
- F. Mandl: *Wien Klin. Wchnschr.* 38:1343, 1925.
- F. Mandl: *Zentralbl. f. Chir.* 53:260, 1926.
- F. Mandl: *Zentralbl. f. Chir.* 56:1739, 1929.
- A. D. Marenzi, R. Gerschman: *Comp. Rend. Soc. de biol.* 117:56, 1934.
- D. Marine: *Proc. Soc. Exper. Biol. a. Med.* 11:117, 1914.
- J. L. Mathies, O. H. Gaebler: *Endocrinology* 45:129, 1949.
- J. L. Mathies, O. H. Gaebler, L. Palm: *Endocrinology* 45:480, 1949.
- P. Mazzocco: *Compt. Rend. Soc. de biol.* 97:594, 1927.
- J. Mellgren: *Acta path. et microbiol. Scand.* 20:693, 1943.
- J. Mellgren, G. Lundh: *Acta path. et microbiol. Scand.* 23:330, 1946.
- C. Mentha: *Schweitz. Ztschr. f. Path. u. Bakt.* 4:209, 1941.
- B. B. Migicovsky, A. R. G. Emslie: *Arch. Biochem.* 20:325, 1949.
- St. M. Milcou, M. Pitis: *Bull. Acad. med. Roumanic* 12:230, 1942.

- St. M. Milcou, M. Pitis: *Acta Endocrin. Bucuresti.* 14:90, 1948.
- E. S. Miller, L. R. Evans: *New England J. Med.* 227:949, 1942.
- E. P. Monahan, S. Freeman: *Am. J. Physiol.* 142:104, 1944.
- A. F. Morgan, L. Kimmel, R. Thomas, Z. Samisch: *J. Biol. Chem.* 106:531, 1934.
- R. E. Mosher, A. J. Boyle, E. J. Bird, S. D. Jacobson, T. M. Batchelor, L. T. Iseri, G. B. Myers: *Am. J. Clin. Path.* 19:461, 1949.
- C. M. Plum: *Acta physiol. scand.* 6:289, 1943.
- Möllendorff: *Handbuch B.* 1:2, 1929.
- I. T. Nathanson, A. M. Bruces, R. W. Rawson: *Proc. Soc. Exper. Biol. a. Med.* 43:737, 1940.
- A. H. Neufeld, J. B. Collip: *Endocrinology* 30:135, 1942.
- B. Norberg: *Acta physiol. scand. suppl.* 14, 1942.
- E. Norinder: *Diss. Uppsala* 1939. *Uppsala läkareförenings Förhandl. N. F. Bd XLV häfte* 5—6.
- E. H. Norris: *Arch. Path.* 42:261, 1946.
- H. C. Olsen: *Diss. København* 1934.
- M. D. Overholser: *Anat. Rec.* 41:303, 1928.
- A. M. Pappenheimer, S. L. Wilens: *Am. J. Path.* 11:73, 1935.
- H. M. Patt, E. Wallerstein, A. B. Luckhardt: *Proc. Soc. Exper. Biol. a. Med.* 49:580, 1942.
- H. M. Patt, A. B. Luckhardt: *Endocrinology* 31:384, 1942.
- R. M. Perlman: *J. Clin. Endocrinology* 4:483, 1944.
- R. M. Perlman: *Arch. Path.* 38:20, 1944.
- G. Pincus, K. V. Thimann: *New York* 1948.
- A. Pope, J. C. Aub: *New England J. Med.* 230:698, 1944.
- L. I. Pugsley, E. M. Anderson: *Am. J. Physiol.* 109:85, 1934.
- L. I. Pugsley, E. M. Anderson: *Biochem. J.* 28:1313, 1934.
- L. I. Pugsley: *Am. J. Physiol.* 113:108, 1935.
- L. I. Pugsley, J. B. Collip: *Biochem. J.* 30:1274, 1936.
- M. R. Reid, J. Ransohoff: *Am. J. Med. Science* 206:731, 1943.
- O. Riddle, L. B. Dotti: *Science* 84:557, 1936.
- O. Riddle, V. M. Rauch, G. C. Smith: *Endocrinology* 36:41, 1945.
- E. De Robertis: *Anat. Rec.* 78:473, 1940.
- E. De Robertis: *Anat. Rec.* 79:417, 1941.
- J. M. Rogoff, G. N. Stewart: *Am. J. Physiol.* 86:25, 1928.
- J. M. Rogoff: *Science* 80:319, 1934.
- J. A. Rosof: *J. Exper. Zool.* 68:121, 1934.
- G. Rucart: *Arch. d'anat. microscop. morph. expér.* 38:1, 1949.
- E. Rutishauser: *Ann. d'anat. path.* 13:999, 1936.
- J. H. C. Ruyter, H. Neumann: *Biochimica et biophysica acta* 3:125, 1949.
- H. A. Salvesen, G. C. Linder: *J. Biol. Chem.* 58:635, 1923.
- H. A. Salvesen: *Acta Med. Scand.* 83:485, 1934.

- I. Sandström: Uppsala läkareförenings förhandlingar 15:441, 1880.
 Schlagenhauer: Wien Klin. Wchnschr. 28:1362, 1915.
 I. Schour, J. M. Rogoff: Science 83:267, 1936.
 H. Selye: Endocrinology 16:547, 1932.
 H. Selye: Arch. Path. 34:625, 1942.
 H. Selye: Montreal 1948.
 H. A. Shapiro, H. Zwarenstein: J. Exper. Biol. 10:186, 1933.
 H. A. Shapiro, H. Zwarenstein: J. Exper. Biol. 11:267, 1934.
 S. Shapiro, H. L. Jaffe: Endocrinology 7:720, 1923.
 J. H. Shaw, R. O. Greep: Endocrinology 44:520, 1949.
 D. H. Shelling: J. Biol. Chem. a/ 96:195 b/ 96:215, 1932.
 D. H. Shelling, D. E. Asher, D. A. Jackson: Bull. Johns Hopkins Hosp. 53:348, 1933.
 D. H. Shelling, L. Kajdi, L. Guth: Endocrinology 22:225, 1938.
 J. G. Sinclair: Anat. Rec. 80:479, 1941.
 P. E. Smith: J. A. M. A. 88:158, 1927.
 F. H. Snyder, W. R. Tweedy: Proc. Soc. Exper. Biol. a. Med. 47:234, 1941.
 H. C. Stoerk: Proc. Soc. Exper. Biol. a. Med. 54:50, 1943.
 H. C. Stoerk, W. H. Carnes: J. Nutrition 29:43, 1945.
 A. T. Strohl: J. Nutrition 11:275, 1936.
 H. M. Teel, O. Watkins: Am. J. Physiol. 89:662, 1929.
 H. M. Tepperman, M. V. L'Heureux, A. E. Wilhelmi: J. Biol. Chem. 168:151, 1947.
 K. W. Thompson, H. Cushing: Proc. Roy. Soc. /London/ S. B. 115:88, 1934.
 D. L. Thomson, J. B. Collip: Physiological Reviews 12:309, 1932.
 G. W. Thorn, L. L. Engel, H. Eisenberg: J. Exper. Med. 68:161, 1938.
 C. E. Tobin: Am. J. Anat. 65:151, 1939.
 W. R. Tweedy, F. C. Kock: J. Lab. a. Clin. Med. 14:747, 1929.
 W. R. Tweedy, S. B. Chandler: Am. J. Physiol. 88:754, 1929.
 W. R. Tweedy, W. W. Campbell: J. Biol. Chem. 154:339, 1944.
 W. R. Tweedy, M. E. Chilcote, M. C. Patras: J. Biol. Chem. 168:597, 1947.
 N. Törnblom: Nord. Med. 33:661, 1947.
 N. Törnblom: Nord. Med. 41:321, 1949.
 N. Törnblom: Acta Endocrin. suppl. 4, 1949.
 G. Vassale, F. Generali: Arch. ital. de Biol. 33:154, 1900.
 L. Vermetti: Ormoni 1:737, 1939.
 C. Verstraeten, O. Vanderlinden: Mém. couron. Acad. roy. de méd. de Belg. 13:1, 1894.
 S. O. Waife: Am. J. Med. Science 218:624, 1949.
 E. W. Wallace: J. Pharmacol. 54:161, 1935.
 W. White: Proc. Roy. Soc. London ser. B. 64:114, 1933.

- R. M. Wilder, J. L. Johnson: J. A. M. A. 96:1987, 1931.
R. M. Wilder, G. M. Higgins, C. Sheard: Ann. Inst. Med. 7:1059,
1933/34.
Å. Wilton: Acta paed. 17:72, 1934.
Å. Wilton: Kimpton, London 1937.
Å. Wilton: Acta path. et microbiol. Scand. 23:1, 1946.
T. R. Wood, W. F. Ross: J. Am. Chem. Soc. 64:2759, 1942.
M. O. Young, B. Halper: Arch. Path. 44:628, 1947.
B. Zondek: Lancet 2:842, 1936.

APPENDIX WITH TABLES

Table I.
Normal rats, new-born.*)

Parathyroids in cu. mm.	$M \pm \epsilon_m$	No. of cases
pr. Gm. of birth weight	0.00230 ± 0.00004	19

*) From Hultquist, Engfeldt, Acta endocrinol. 3, 365, 1949.

Table II.
50-day-old male rats, normal diet.

Weight in gm.	Parathyr. volume in cu. mm.	Parathyr. volume per gm. of body weight	Testes weight in mg.	Testes mg. per gm. of body weight	Adre- nals in mg.	Adrenals mg. per gm. of body weight	Kidneys, weight in mg.
60	0,1072	0,00179	1360	22,67	17	0,283	750
50	0,1096	0,00219	910	18,20	14	0,280	670
60	0,0909	0,00151	1065	17,75	17	0,283	680
50	0,0909	0,00182	790	15,80	16	0,320	605
60	0,0656	0,00109	950	15,83	17	0,283	660
60	0,793	0,00132	880	14,67	18	0,300	665
55	0,0909	0,00165	459	8,35	18	0,327	670
60	0,0884	0,00147	726	12,10	22	0,367	770
60	0,1028	0,00171	420	7,00	21	0,350	580
55	0,1478	0,00269	1200	21,82	20	0,364	810
55	0,0805	0,00146	812	14,76	23	0,418	790
M	0,0958	0,00170	870	15,36	18	0,325	695
ϵ_m	0,0065	0,000132	85,1	1,50	0,8	0,014	22,4

Table III.
65-day-old male rats, normal diet.

Weight in gm.	Parathyr. volume in cu. mm.	Parathyr. volume per gm. of body weight	Testes weight in mg.	Testes mg per mg. of body weight	Adre- nals in mg.	Adrenals mg. per gm. of body weight	Kidneys. weight in mg.
90	0,0670	0,00074	1280	14.22	28	0.311	1155
100	0,1456	0,00146	635	6.35	38	0.380	1085
110	0,1124	0,00102	1310	11.91	36	0.327	1350
100	0,1470	0,00147	1350	13.50	35	0.350	1480
100	0,1004	0.00100	1000	10.00	30	0.300	1100
105	0,0962	0,00092	1680	16.00	35	0.333	1050
110	0.1148	0,00104	850	7.73	34	0.309	1055
100	0,1031	0,00103	1500	15.00	30	0.300	1000
95	0,1342	0,00141	1455	15.32	25	0.263	940
100	0,1152	0.00115	1066	10.66	30	0.300	910
100	0,1121	0,00112	1200	12.00	32	0.320	1220
M	0,1135	0,00112	1211	12.06	32	0.318	1122
ϵ_m	0,0069	0,000070	91,6	0.95	1,1	0,009	51,4

Table IV.
80—90-day-old male rats, normal diet.

Weight in gm.	Parathyr. volume in cu. mm.	Parathyr. volume per gm. of body weight	Testes weight in mg.	Testes mg. per gm. of body weight	Adre- nals in mg.	Adrenals mg. per gm. of body weight	Kidneys. weight in mg.
145	0,1122	0,00077	2160	14,90	36	0,248	1680
150	0,1568	0,00105	1705	11,37	30	0,200	1700
140	0,1153	0,00082	1450	10,36	31	0,221	1750
140	0,1169	0,00083	1450	10,36	30	0,214	1350
140	0,1199	0,00086	960	6,86	30	0,214	1540
145	0,1141	0,00079	980	6,76	35	0,241	1510
150	0,1507	0,00100	680	4,53	32	0,213	1500
130	0,1255	0,00097	710	5,46	35	0,269	1505
155	0,1988	0,00128	530	3,42	35	0,226	1700
150	0,1086	0,00072	650	4,33	36	0,240	1760
160	0,1218	0,00076	2100	13,13	32	0,200	1520
160	0,1498	0,00094	2300	14,38	30	0,188	1700
150	0,1541	0,00103	2050	13,67	31	0,207	1550
150	0,0997	0,00066	2030	14,53	27	0,180	1500
M	0,1317	0,00089	1411	9,58	32	0,219	1590
ϵ_m	0,0072	0,000044	173	1,13	0,8	0,007	32,7

Table V.
150—200-day-old male rats, normal diet.

Weight in gm.	Parathy. volume in cu. mm.	Parathy. volume per gm. of body weight	Testes weight in mg.	Testes mg. per gm. of body weight	Adre- nals in mg.	Adenals mg. per. gm. of body weight	Kidneys. weight in mg.
210	0,1096	0,00052	2705	12,88	51	0,243	1820
240	0,0881	0,00037	2795	11,65	60	0,250	2010
215	0,0855	0,00040	2210	10,28	38	0,177	1880
250	0,1439	0,00058	2780	11,12	42	0,168	1990
250	0,1615	0,00065	2820	11,28	56	0,224	2200
180	0,0917	0,00051	2350	13,06	40	0,222	1760
210	0,1965	0,00094	2675	12,74	43	0,205	1930
215	0,1481	0,00069	2240	10,42	51	0,237	1980
180	0,1600	0,00089	2200	12,22	41	0,228	1830
180	0,1088	0,00060	2250	12,50	34	0,189	1870
180	0,1776	0,00099	2050	11,39	35	0,194	1710
180	0,1520	0,00084	1750	9,72	35	0,194	1850
175	0,1091	0,00062	2450	14,00	30	0,171	1550
220	0,1551	0,00071	2250	10,23	48	0,218	1810
225	0,1507	0,00067	1720	7,64	50	0,222	1510
180	0,1441	0,00080	2380	13,22	36	0,200	1800
M	0,1364	0,00067	2352	11,52	43	0,209	1844
ϵ_m	0,0083	0,000046	85,8	0,40	2,2	0,006	42,5

Table VI.
80—90-day-old male rats treated with calf pituitary,
normal diet.

Weight in gm.	Parathyr. volume in cu. mm.	Parathyr. volume per gm. of body weight	Testes weight in mg.	Testes mg. per gm. of body weight	Adre- nals in mg.	Adrenals mg. per gm. of body weight	Kidneys, weight in mg.
130	0,1319	0,00101	1780	13,69	40	0,308	1400
130	0,1759	0,00135	1750	13,46	55	0,423	1550
130	0,1585	0,00122	1200	9,23	60	0,462	1700
130	0,1518	0,00117	1460	11,23	55	0,423	1450
130	0,1496	0,00115	910	7,00	50	0,385	1150
170	0,1884	0,00111	1450	8,53	56	0,329	1610
150	0,1405	0,00094	1050	7,00	65	0,433	1850
160	0,1615	0,00101	700	4,38	78	0,488	1860
170	0,1677	0,00099	1800	10,59	64	0,376	1910
150	0,1369	0,00091	810	5,40	77	0,513	1720
170	0,2905	0,00171	2055	12,09	69	0,406	1515
175	0,1794	0,00103	1950	11,14	105	0,600	2380
170	0,2212	0,00130	1850	10,88	90	0,529	1760
165	0,1912	0,00116	2280	13,82	85	0,515	2010
140	0,1330	0,00095	1710	12,21	46	0,329	1560
170	0,1742	0,00102	1790	10,53	48	0,282	1650
M	0,1720	0,00113	1534	10,07	65	0,425	1692
ϵ_m	0,0099	0,000050	118	0,73	4,5	0,022	70,6

Table VII.
150—200-day-old male rats treated with calf pituitary, "
normal diet.

Weight in gm.	Parathy. volume in cu. mm.	Parathy. volume per gm. of body weight	Testes weight in mg.	Testes mg. per mg. of body weight	Adre- nals in mg.	Adrenals mg. per mg. of body weight	Kidneys weight in mg.
180	0,1524	0,00085	1900	10,56	55	0,306	1830
180	0,1613	0,00090	2080	11,56	55	0,306	2130
195	0,2011	0,00103	2580	13,23	86	0,441	2650
180	0,1332	0,00074	2000	11,11	155	0,861	2180
220	0,1250	0,00057	2505	11,39	72	0,327	2200
225	0,2206	0,00098	2240	9,95	85	0,378	2450
235	0,1746	0,00074	2450	10,43	75	0,319	2850
220	0,1558	0,00071	2500	11,36	78	0,355	2330
210	0,2271	0,00108	1860	8,86	67	0,319	1780
180	0,3423	0,00190	1630	9,06	75	0,417	2015
210	0,3878	0,00185	2625	12,50	61	0,290	1580
190	0,1824	0,00096	2260	11,89	95	0,500	1730
230	0,3258	0,00142	2400	10,43	55	0,239	2010
220	0,2243	0,00102	2520	11,45	82	0,373	1980
230	0,2625	0,00114	2480	10,78	70	0,304	2370
200	0,2335	0,00117	2290	11,45	56	0,280	1980
M	0,2194	0,00107	2270	11,00	76	0,376	2129
ϵ_m	0,0192	0,000094	73,9	0,28	6,1	0,036	85,2

Table VIII.
Growth hormone 20 u daily, 150—200-day-old male rats,
normal diet.

weight in gm.	Parathyr. volume in cu. mm.	Parathyr. volume per gm. of body weight	Testes weight in mg.	Testes mg. per gm. of body weight	Adre- nals in mg.	Adrenals mg. per gm. of body weight	Kidneys, weight in mg.
200	0,1759	0,00088	2070	10,35	63	0,315	1850
200	0,1336	0,00067	1850	9,25	55	0,275	1700
200	0,1440	0,00072	1720	8,60	58	0,290	1640
200	0,1379	0,00069	2060	10,30	54	0,270	1750
200	0,1604	0,00080	1750	8,75	57	0,285	1640
190	0,1502	0,00079	2110	11,11	38	0,200	1850
200	0,2371	0,00119	2750	13,75	40	0,200	2100
200	0,1428	0,00071	2540	12,70	41	0,205	1840
170	0,1482	0,00087	2100	12,35	42	0,247	1680
180	0,1627	0,00090	2410	13,39	38	0,211	1900
170	0,1456	0,00086	2040	12,00	42	0,247	1720
M	0,1580	0,00083	2127	11,14	48	0,250	1788
ϵ_m	0,0086	0,000044	97,2	0,55	2,8	0,012	41,5

Table IX.
Hypophysectomized 150—200-day-old male rats, normal diet.

weight		No. of days	Parathyr. volume in cu. mm.	Parathyr. per gm. of body weight (op.)	Parathyr. vol. per gm. body weight (dead)	Testes weight in mg.	Testes mg. per gm. body weight (dead)	Adrenals in mg.	Adrenals per gm. body weight (dead)	Kidneys, weight in gm.
At op.	At death									
220	140	23	0,1693	0,00077	0,00121	400	2,86	14	0,100	1460
200	115	21	0,1136	0,00057	0,00099	280	2,43	12	0,104	910
220	175	14	0,1474	0,00067	0,00084	790	4,51	24	0,137	1350
210	160	13	0,1079	0,00051	0,00067	1000	6,25	20	0,125	1250
200	150	18	0,1154	0,00058	0,00077	950	6,33	18	0,120	1450
240	150	20	0,0949	0,00040	0,00063	610	4,07	12	0,080	1300
250	120	23	0,0927	0,00037	0,00077	350	2,92	9	0,075	1160
230	120	24	0,1093	0,00048	0,00091	340	2,83	7	0,058	1150
260	160	24	0,1265	0,00049	0,00079	400	2,50	10	0,062	1250
200	160	18	0,0838	0,00042	0,00052	750	4,69	15	0,094	1240
250	200	13	0,1576	0,00063	0,00079	1070	5,35	12	0,060	1320
M		19	0,1199	0,00054	0,00081	631	4,07	14	0,092	1258
$\bar{x}$ in			0,0083	0,000037	0,000056	88,6	0,44	1,5	0,008	46,2

Table X.
Completely hypophysectomized 150—200-day-old male rats, normal diet.

Weight		Time		Blood phosphorus mg. %	Amount of urine ml/24 hrs.		Urine phosphorus mg. per 24 hrs.		Blood cal- cium %	Plasma protein %	Adre- nals weight in mg.	Testes, weight in mg.			
At op.	At death	Op	Death												
195	140	14/3	1/4	4,7/8*	4,3/18/	18/4/	13/10/	15/16/	7,4	3,2	3,7	10,5	6,1	6,5	450
155	120	15/3	1/4	4,5/8/	4,2/17/	5/5/			2,9			11,1	6,8	13,0	460
205	145	10/3	4/4	6,1/8/	5,5/25/	29/5/	11/19/		4,1	5,4		9,7	7,0	11,9	295
170	125	10/3	4/4	4,8/8/	4,8/25/	12/5/	7/12/		4,4	3,0		9,9	6,4	13,0	262
175	125	11/3	4/4	5,2/9/	4,8/24/	24/4/	10/18/		6,2	2,3		10,7	6,2	10,0	233
195	145	14/3	5/4	5,0/7/	4,6/22/	28/3/	14/9/	21/16/	5,8	1,3	5,4	10,7	7,1	10,8	290
185	140	14/3	5/4	5,2/6/	4,0/22/	23/3/	10/9/	11/16/	7,6	3,2	2,8	9,5	7,4	12,0	348
155	135	14/3	5/4	5,0/6/	4,1/22/	11/3/			3,1			11,3	6,8	10,0	290
190	150	15/3	5/4	5,3/7/	4,6/21/	24/3/	15/15/		6,8	2,3		10,7	6,9	10,0	410
M				5,1	4,5	19			5,4			10,5	6,7	10,8	338
ϵ_m				0,15	0,15	2,8			0,60			0,21	0,14	0,67	27,9

*) The figures within brackets = the number of days after operation when the specimen was taken.

Table XI.
Hypophysectomized except for the anterior lobe. 150—200-day-old male rats, normal diet.

Weight		Time		Blood phosphorus mg %	Amount of urine ml./24 hrs.	Urine phosphorus mg. per 24 hours	Blood calcium mg. %	Plasma protein %	Adrenals weight in mg.	Testes weight in mg.
At op.	At death	Op.	Death							
180	170	14/3	1/4	4,3/8/*	42/4/	13,9	10,7	7,1	42,0	1940
185	190	15/3	1/4	4,6/8/	26/3/	6,7	9,9	6,8	61,0	2360
150	170	10/3	4/4	5,9/8/	27/5/	6,4	10,3	6,8	40,7	1130
180	180	11/3	4/4	6,4/8/	38/5/	8,2	11,1	6,4	41,5	2030
220	230	11/3	4/4	5,8/8/	59/5/	12,0	10,7	7,2	53,2	2320
170	180	11/3	4/4	6,1/8/	36/5/	4,6	10,5	7,6	37,8	2110
210	185	11/3	5/4	7,1/8/	29/6/	5,5	10,7	6,1	44,0	1140
230	245	11/3	5/4	6,6/8/	60/6/	15,9	10,2	7,7	48,7	2205
190	220	16/3	6/4	6,0/8/	12/5/	5,8	10,5	6,7	45,0	2200
M				5,9	37	8,8	10,5	6,9	46,0	1937
ϵ_m				0,30	5,2	1,37	0,12	0,17	2,42	158

*) The figures within brackets = the number of days after operation when the specimen was taken.

